Biological
& Medical
Serials

Public health bulletin
no.38-39

CONNAUGHT ANTITOXIN LABORATORIE
RESEARCH DIVISION

TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

PUBLIC HEALTH BULLETIN No. 38

GENERAL OBSERVATIONS ON THE BIONOMICS OF THE RODENT AND HUMAN FLEAS

Library
School of Hygiene
University of Toronto

BY

MAURICE B. MITZMAIN, M. S.
Assistant, Plague Laboratory, United States Public Health and Marine-Hospital Service

PREPARED BY DIRECTION OF THE SURGEON-GENERAL

LIBRARY
SEP 5 1963
UNIVERSITY OF TORONTO

WASHINGTON
GOVERNMENT PRINTING OFFICE
1910

TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

PUBLIC HEALTH BULLETIN No. 38

GENERAL OBSERVATIONS ON THE BIONOMICS OF THE RODENT AND HUMAN FLEAS

Library
School of Hygiene
University of Toronto

BY

MAURICE B. MITZMAIN, M. S.
Assistant, Plague Laboratory, United States Public Health and Marine-Hospital Service

PREPARED BY DIRECTION OF THE SURGEON-GENERAL

Library
School of Hygiene
University of Toronto

WASHINGTON
GOVERNMENT PRINTING OFFICE
1910

RA
421
P78
no 38-39
cop. 2

LIBRARY
APR 26 1965
UNIVERSITY OF TORONTO

977722

GENERAL OBSERVATIONS ON THE BIONOMICS OF THE RODENT AND HUMAN FLEAS.

By Maurice B. Mitzmain, M. S., Assistant, Plague Laboratory, United States Public Health and Marine-Hospital Service, San Francisco, Cal.

It is desired in this paper to convey an insight into the habits and metamorphoses of the California species of fleas commonly found on rats, ground squirrels, and man. Numerous requests from medical officers for information of this nature are regarded as sufficient justification for the compilation of these notes. The paper is preliminary in character, as the studies are still in progress.

THE MATERIALS USED FOR FLEA DEVELOPMENT.

We have made an effort to rear fleas in the laboratory by attempting to duplicate conditions found to exist in nature. The human flea, *Pulex irritans* Linn., was found to develop very satisfactorily in a medium composed of floor sweepings taken from cracks in the floor. The squirrel fleas and rat fleas were found to develop well in material taken from the nests of their respective hosts. We have experienced no difficulty in raising these parasites by placing animals covered with fleas in cages with a bedding of sawdust; and the only provision necessary for complete development was the addition of fresh sawdust to prevent the accumulation of too much moisture.

An experiment was made to determine in what nesting material fleas away from the host would survive longest. For this purpose we tested various materials shown in the following table:

Length of life in various nesting materials.

CERATOPHYLLUS ACUTUS BAKER.

[M=male; F=female.]

Removed from host.	Dry sand with squirrel droppings.	Moist sand from squirrel nest.	Dry sand from squirrel nest.	Sawdust moistened with horse serum.	Dry sawdust.	Moistened sawdust with wheat grains.
2 days	5 M.; 5 F.	10 F.	10 F.	4 M.; 6 F.	4 M.; 6 F.	4 M.; 6 F.
7 days	2 M.; 5 F.	4 F.	All dead.	1 M.; 3 F.	4 F.	1 M.; 5 F.
9 days		1 F.		2 F.	2 F.	1 M.; 4 F.
10 days	2 M.; 5 F.	All dead.		All dead.	All dead.	1 M.; 4 F.
14 days	1 M.; 1 F.					1 M.; 3 F.
16 days	1 M.; 1 F.					
17 days	All dead.					1 M.; 3 F.
20 days						3 F.
25 days						1 F.
26 days						All dead.

The controls, 5 males, 5 females, were all dead on or before the seventh day.

In the medium of moistened sawdust mixed with a few wheat grains it was found that the wheat sprouted in the sawdust and held sufficient moisture to provide a suitable condition for larvæ as well as for adults. The mould which formed in a short time did not seem to affect the insect life.

It is seen that the fleas did not fare well in the medium of dry sand. The sand was mixed with clay dust, which would rise whenever the fleas hopped in the vial. As a consequence death resulted, presumably by stoppage of the spiracles.

It will be seen from a survey of this table that fleas taken from the natural host may be kept alive without food for a considerable time. The medium of moistened sawdust with a few grains of wheat seemed to answer the moisture requirements for flea life.

This material was used to determine the longevity of squirrel fleas reared from cocoons in the laboratory. The adults had emerged October 4, 1909, and were transferred on the same day to an open museum jar, supplied with a layer of sawdust and moistened wheat grains. Twenty specimens, 8 males and 12 females, were used in this experiment. On the twenty-eighth day 1 male and 8 females were alive. The male survived until the thirty-eighth day, when only a single female remained. This female lived until January 25, 1910, a period of eighty-one days from the time of emergence.

The virility of these fleas was shown when a male and a female were applied to the hand of the experimentor and fed on the twenty-seventh day. The two fed fleas were excluded from the experiment after the feeding.

LOCOMOTION.

The only literature that has come to our notice on the jumping powers of fleas appears in the "Reports of the advisory committee on plague investigations in India" (Journal of Hygiene, 1906, vol. 6, p. 464). Here we have a note: "It had previously been found that a rat flea could not hop farther than 5 inches." The species in question in these experiments was the *Læmopsylla cheopis* Roth, which constitutes 99 per cent of the fleas found on rats in India.

It seemed desirable to learn the jumping abilities of the common California species. An attempt was made to determine the distance upon a horizontal plane, as well as the height that the insect could jump. In the broad jump a few experiments were conducted with *P. irritans*, the most active of our California fleas. In this species we have found the jumping distance varies considerably with the nature of the container from which the insect is observed, eg., a foot-

hold of wood enables the flea to jump a greater distance than one of glass. Since the *irritans* is found to predominate on floors of houses, a surface of wood was selected as the footing in our tests. In one experiment five specimens were permitted to jump at will and the jumps of each were recorded. The mean average of ten jumps of each specimen gave a distance of 7.3 inches. The longest jump recorded was 11 inches; this was made by a female. In another experiment a female, which was starved for five days prior to the test, made four jumps, respectively, 10.5, 11, 12, and 13 inches, averaging $11\frac{5}{8}$ inches. Thirteen inches was the longest jump recorded on a horizontal plane. A jump of $15\frac{7}{8}$ inches was made downward at an angle of 30 degrees.

This last specimen was then permitted to feed fifteen minutes on the arm of an attendant, after which it was carefully returned to the container and its jumps for five minutes recorded. The longest jump after feeding was 12 inches.

The jumping powers of squirrel and rat fleas were tested in this manner: Three hundred and seventy-five live squirrel fleas (*Ceratophyllus acutus*) and 150 live rat fleas (*C. fasciatus* Bosc. and *L. cheopis*) were segregated into species and placed in 15 open specimen vials in a water bath and left undisturbed for two days. At the end of the period the vials were examined, the water bath and the surroundings being carefully inspected. No fleas could be detected in the water bath or in the vicinity. The fleas in the vials were counted, the original number being present. They were apparently as active as when removed from their hosts. The containers were cylindrical vials $3\frac{3}{8}$ inches in height and $1\frac{3}{16}$ inches in diameter. The same test was tried in open shell vials of the next size smaller ($3\frac{1}{8}$ by 1 inch), and the fleas were found jumping into the water bath.

L. cheopis, the rat flea, was tried for its jumping powers. It had been previously observed that members of this species were unable to leap out of open shell vials $3\frac{3}{8}$ inches in height. It was thought desirable to permit the flea greater latitude, making the trial fairer and more practical. Twenty fleas (8 males and 12 females) were placed in a rectangular porcelain dish, the dimensions of which were $11\frac{1}{8}$ inches by $7\frac{3}{8}$ inches by $2\frac{1}{4}$ inches deep. A light sprinkling of moist sand was placed in the bottom of the dish to provide a firm footing and the sides of the dish were extended to the height of $4\frac{1}{2}$ inches by inclosing the container entirely by strips of "tangle-foot" paper. At the end of twenty-four hours the dish with the fleas was examined and it was observed that several fleas had leaped high enough to become entangled in the adhesive paper. The position of these was noted; the distance from the bottom of the pan was

measured. The entangled insects were pried off, the sticky material dissolved with alcohol, and the insects examined microscopically. Five specimens were collected, two males and three females. The locations on the adhesive paper relative to the base of the dish were as follows: One female $2\frac{1}{2}$ inches, 1 female $3\frac{1}{8}$ inches, 1 female $2\frac{9}{16}$ inches; 1 male $2\frac{5}{8}$ inches, 1 male $3\frac{1}{16}$ inches.

A census of the fleas remaining in the bottom of the dish gave 6 males and 9 females, showing that none jumped over the surrounding paper, and that all jumping over $2\frac{1}{2}$ inches were embedded in the adhesive paper.

The jumping powers of *P. irritans* were further tested, 20 fleas of this species being placed in a rectangular museum jar, the inside dimensions of which were $2\frac{1}{4}$ by $5\frac{1}{2}$ by $7\frac{3}{4}$ inches deep. The glass lid of the jar was coated with "tangle foot" and the upper 2 inches of the jar were coated with the same material. The following morning the jar was examined and two specimens were seen embedded in the "tangle foot" on the lid. The inside height of the jar being $7\frac{3}{4}$ inches from the base gives then this height as the perpendicular jump.

Concerning the flea's ability to walk upward on glass, we have noted that they can not climb to any considerable distance. Approximately 100 counts were taken, the greatest distance observed being three-quarter inches. The climb appears very laborious, and in all cases the flea dropped to the bottom of the jar after a few efforts.

The style in which rodent fleas walk through the pelage of the host is worthy of comment. They seem to shuffle along on the flat side of the tarsi, parting the dense hairs into furrows which close over the active insect with a wavy motion. When on the animal they seldom hop about, unless disturbed or unless the host snaps at them or scratches when unusually annoyed. They hop freely when jumping from the animal to the ground or vice versa, or from one host to another. When a flea is cornered; that is, when it experiences difficulty in passing an obstruction, it proceeds like a swimmer using the side stroke. The parasite drops to its side and locomotion ensues by a vigorous sweeping movement of the legs, almost entirely by the use of the hairs and spines, especially through the medium of the powerful spines of the tibia effecting a progressive movement while it ambles along on the flat side in a striking manner. The spines of the leg seem to be peculiarly adapted for this side motion. It is in this fashion that the nimble parasite manages to become so very elusive. We refer especially to the *P. irritans*. This may be tested by holding a live flea between the thumb and forefinger, and unless you chance to be a flea trapper of long and painful experience the ingenious parasite will surely escape.

CONSIDERATION OF COLOR ATTRACTION.

A little experimental evidence has been obtained on the question of attraction toward color in animals. It is the prevailing opinion that white animals attract the greatest number of fleas. A few tests of color attraction are cited here. The material used in the first experiment was six guinea pigs showing extreme variations of color. Three of these were pure white in color and three were dark brown and black. These were placed in a large cage, the bedding of which was infested with squirrel fleas. After forty-eight hours the guinea pigs were removed simultaneously and a census of the fleas on the individual animals was made immediately. Fleas and animals were anæsthetized at the same time, the white guinea pigs yielding, respectively, 10 fleas, 5 fleas, and 11 fleas. The black guinea pigs yielded 11, 7, and 4 fleas; the last number came from a mixed black and white guinea pig. A summary of the fleas from the three white guinea pigs gave 26 fleas, or an average of about 9 each; the two dark guinea pigs giving 18, averaging 9 each; and the black and white giving 4 fleas, all of which were of the species *C. acutus*, the common squirrel flea.

A second experiment was as follows: Four guinea pigs were placed in a large container which was swarming with squirrel fleas. After three days the animals were removed as before, and a census of the parasites was taken; one brown and black guinea pig yielded 89 fleas; another brown and black guinea pig yielded 57 fleas; a pure white guinea pig yielded 57 fleas; and the other white guinea pig yielded 60 fleas. These guinea pigs were kept together in the open vessel under the same conditions of light and temperature.

One experiment was conducted by using guinea pigs as traps for fleas in an infested house. The results shown are relatively meager, due to the previous use of adhesive paper for flea trapping. In this test six guinea pigs were permitted to wander for twenty-four hours through the basement of the house. The fleas collected from the guinea pigs were *P. irritans*, found on the hosts as follows:

Color of animal.	Number of fleas.
Black guinea pig	1
White guinea pig	1
Black guinea pig	1
White guinea pig	1
Black guinea pig	0
White guinea pig	0

It is obvious from the meager evidence presented in these experiments that color does not exert the influence generally claimed for it.

The white animals are no more attractive to the fleas we have used than are the dark-colored ones.

TRAPPING OF FLEAS WITH MEAT AS A BAIT.

An idea prevails that fleas can be attracted and trapped on account of their predilection for the odor of fresh meat. This idea was put to test in an experimental way. On the 23d day of August, 1909, during a season of the year in which fleas were extremely abundant, a vacant house, which was found to be flea infested, was chosen for the experiment. The fleas were so abundant that when two attendants, who had occasion to enter the first floor of the dwelling, remained for a period of five minutes and emerged covered with fleas, approximately 200 fleas were taken from their clothing and persons. The materials used for the experiment were 12 sheets of "tangle-foot" fly paper, distributed in pairs among three rooms of the house. One sheet of each pair was supplied with a small fresh piece of cow's liver. Care was taken to distribute the sheets in such a manner that the influence of light would be the same for each pair of sheets in the series. The sticky fly papers were left undisturbed for a period of three days, then collected and examined.

A similar experiment was conducted at a later date, December 5, 1909, when San Francisco dwellings were still flea infested. The sheets of fly paper were left in the basement of a house for twenty-four hours. The fleas in both experiments were removed by dissolving the "tangle-foot" in alcohol. The parasites, which were identified as *Pulex irritans*, were distributed on the sheets as follows:

Total number of fleas trapped.

Pair.	Experiment 1.		Experiment 2.	
	Adhesive paper with meat.	Adhesive paper without meat.	Adhesive paper with meat.	Adhesive paper without meat.
1	0	0	2	4
2	2	2	1	3
3	2	1	6	4
4	0	3	47	49
5	6	12	1	0
6	4	1	4	1
Total	14	19	61	61

The table shows that according to our experience, though the data are limited, meat used as a bait under the conditions stated does not exert any attraction for fleas.

A third experiment of the same character as the two foregoing was conducted in a house which was infested with both *P. irritans*

and *Ctenocephalus canis* Curtis. The results show that the count for each species was relatively about the same.

Pairs.	Experiment 3.			
	Adhesive paper with meat.		Adhesive paper without meat.	
	Pulex irritans.	Ct. canis.	Pulex irritans.	Ct. canis.
1	82	23	122	58
2	5	1	6	0
3	98	39	90	42
4	42	12	39	14
5	123	64	120	79
6	49	10	60	14
Total	399	149	437	207

In all of the foregoing "tangle-foot" trap experiments, although care was taken to place the meat on the edges as well as on the middle of the sticky paper, only one flea (*Ct. canis*) was found on the meat used as a bait.

EGG LAYING.

Mammalian blood appears essential to fleas for the performance of the normal functions of copulation and oviposition. In our experience insects kept constantly in jars and reared from cocoons, never having been fed upon a host, have not been observed to copulate or oviposit. In 30 specimens of *P. irritans* taken from a house which had been vacant for six weeks we found, after three days' observation, that the fleas were perfectly healthy, and although females predominated, no eggs were found at the end of this period. As a control, 6 females of this species collected from human hosts when kept in separate vials laid eggs normally, depositing from 5 to 12 in each instance.

Four experiments with *C. acutus*, the squirrel flea, have given results similar to the foregoing, namely, that this species when reared from cocoons and kept starved in jars at room temperature has not been observed to mate or lay eggs.

In 25 paired specimens of several species kept under observation it appears that the male does not long survive the act of mating. It dies even before the female has laid its first batch of eggs.

When fertile females are kept under artificial conditions the eggs are laid in one laying in a period beginning two hours after copulation and extending to a maximum of thirty-six hours. When deprived of food the female has never been observed to oviposit after this length of time.

We may here note that when used experimentally the female is invariably longer lived. Experiments to determine the length of life with human blood diet show that female fleas of all species outlive the male by several weeks. This is doubtless true also under natural conditions, where we find in collecting fleas from the host that the females predominate markedly.

The eggs require optimum conditions of temperature and moisture for hatching. They have never been found on the host except in one instance. In this case a dog was used in the laboratory for supplying fresh fleas; this was done by placing the canine on sheets of paper which in a few hours were littered with a large number of flea eggs. These were laid loosely on the host by the fleas, the eggs falling to the paper, where they were collected. Flea eggs have never been found on man and, if present, would not hatch under normal conditions of the body temperature. We have found that keeping eggs in the incubator at blood heat is sufficient to prevent hatching. If the eggs were laid on the host we would certainly expect to find them on the squirrel, on which animal fleas are most abundant. We have taken from two squirrels (in nature), respectively, 225 *C. acutus* and 376 of the same species; but in no instance even where fleas are present in such large numbers have we been able to find eggs even after carefully combing the host. The *C. acutus* is by all means the best criterion in this matter, since we have found that it lays more eggs than any other of the rodent fleas.

The eggs are laid singly, often appearing in small clusters. They may be viscous as in *P. irritans*, *C. fasciatus*, *L. cheopis*, *C. acutus;* or dry as in *Ct. canis* and *Cten. musculi*. The former adhere to the medium in which they are laid, and the eggs of the last two species are laid loosely, so that they roll about when the vial containing them is shaken.

Eggs may be laid while the insect is still under the influence of an anesthetic, when covered by a glass slip, and when exposed to strong sunlight. It appears to be the first impulse for the female to lay its eggs when removed from the host and placed under artificial conditions. The great majority of the eggs obtained were laid on the first day, beginning almost immediately after the fleas were captured. It is a common observation that many females with their abdomens distended lay their eggs as soon as the vial is closed over them. The number of eggs laid at one laying by different species varies from 3 to 18, the rat fleas averaging 6 and the squirrel fleas lay as many as 18.

THE PROCESS OF HATCHING.

C. ACUTUS.

Six eggs laid while the females were kept under observation were examined from time to time until hatched seven to nine days later.

One egg was observed microscopically during the entire process; the other five eggs used as controls were examined occasionally.

On the seventh day of incubation at room temperature the premonitory signs of hatching were discerned in a very faint rising and falling of the exochorion (outer shell layer) on one side of the shell. During the night, seven hours later, the movement grew more extensive, the pulsations becoming quite pronounced, causing the egg to shift slightly from its position. On the morning of the eighth day a deep gash was observed in the side of the egg. The gash is made by the egg opener, a wedge-shaped, horny, claw-like structure on the dorsal side back of the head of the embryo. This is operated so as to hew through the resistant shell by a series of slits or gashes. The initial gash increases slowly in length, encircling the egg within an hour.

In the course of a few hours seven successive gashes are made. The location of these is quite constant—two on each side of the egg and three at the center, the middle of the central ones being the most distinct. The young embryo produces these slits through the chorion (inner skin of the shell) by turning with its dorsal side against the shell, striking with the egg pick, first against the base of the egg, rising on its hind prolegs and, striking rapidly as it climbs upward. Prior to each stroke the egg pick is poised deliberately, the weight of the head accelerating the blow; this is augmented occasionally by a lateral shaking of the head.

When the last slit encircles the shell the embryo is at the most active stage; it effects a complete rotation in its shell at intervals of ten minutes. The gashes make the shell appear as though cut into ribbons. The rotary movement gradually subsides until a complete turning consumes twenty minutes, and almost imperceptibly there follows a lull. An inactive condition prevails for a period of about seventeen hours. After the resting period it appears that the egg pick is no longer functional; no new gashes are made, the embyro seeming contented to concentrate its energies against the middle gash. This is enlarged by a puffing and dilating of the head; through the semitransparent shell is seen a constant bubbling and a subsequent dilatation of the cuticle.

On the third day of the hatching process a strong movement of the embryo gives decided evidence of the progress of hatching. Immediately beyond the egg pick a triangular slit appears through which bubbles emerge to the surface of the exochorion; thus the amnion (the embryonic cuticle) makes its initial appearance through a crack in the exochorion of the shell.

The amnion splits longitudinally, adhering to the chorion and bulging out as the insect struggles. It is pushed out of the chorion as the abdominal segments are projecting. The emergence is fur-

thered by the young larva pressing the head against its tail, causing the middle of its body to bulge through the central gap of the shell. The amnion becomes noticeably darker as it is exposed to the air; it is now a light brownish-yellow.

The pressure of the body against the shell forms nearly a round hole through which the larva eventually emerges. The opening is enlarged by pressure of the head against the tail, raising the body like a hoop, causing an arch or a hump to appear with dorsal side outward.

The amnion sheds slowly on either side from the middle of the arched abdomen ventrally and with a movement of fluid beneath, it cracks across the abdomen, peeling and wrinkling as the segments telescope. With a final vigorous bubbling and wrinkling the amnion sheds off, the moulted skin falling on either side, exposing the quiet colorless cuticle of the young insect, roughly wrinkled and bristling with slender hairs or setæ. These hairs which at first appear transparent turn grayish when exposed to the air.

When the tail of the larva has been torn loose from the shell, the head and thorax are still imprisoned within the egg, necessitating a maneuvering by waiving its tail in the air, twisting and squirming while standing on its head. The abdomen doubles up ventrally and finally the young larva supported on its tail extricates the head by a violent shaking. When the head is torn from its fastenings, it is found that the amnion has been holding it within the shell. The adhering membrane is cast out when the shell is shaken off. The larva has now fully emerged, the colorless cuticle has turned grayish and the slender threadlike bristles have assumed an irridescent hue

INFLUENCE OF TEMPERATURE ON EGG DEVELOPMENT.

In a series of experiments in which fleas, *L. cheopis*, were subjected to various temperatures, namely, room temperature (75°–80° F.), hot room temperature (88°–90° F.), and cold room temperature (72° F.), the advisory committee on plague investigations in India concluded that there was an optimum temperature at which breeding took place more vigorously than at other temperatures; that a high mean temperature affected the breeding of fleas in restraining the imago from depositing eggs and inhibiting the development of the eggs into larvæ. (Journal of Hygiene, 1908, vol. 8, No. 2, p. 243–244.)

We have repeated in a small way the experiments with the species used by the advisory committee in addition to a few other species.

L. CHEOPIS.

In this experiment, 18 eggs of *L. cheopis* were used to determine the influence temperature exerts on the hatching process. These eggs were laid within a few hours of each other by four females which

were kept with as many males in a glass vial. The eggs were removed within one hour and divided into lots of six.

One lot of six was placed in the cold room, kept slightly below outdoor temperature. One lot was maintained at room temperature in the laboratory. The third lot was kept at the temperature of the incubator. The three lots were placed in glass cell slides, protected with cover slips, and observed at intervals without subjecting them to a change in temperature.

A. Eggs kept at high temperature.—These eggs were placed in the incubator which was regulated at a uniform temperature with a minimum range of 35° C. and a maximum of 37° C. On the third day one egg hatched and the larva almost immediately shriveled and died from the heat. In fact it succumbed in the act of emerging when all parts but the tail were freed.

Not one of the eggs remaining showed evidence of hatching when examined at this time. On the fourth day, there was no evidence of life in the other eggs. They appeared shriveled and dessicated.

B. Eggs kept at room temperature.—These eggs were kept in a covered glass cell on a table in the laboratory. The cell slide was covered so as to be protected from the light. The temperature recorded during the hatching period was 17° C. minimum and 23° C. maximum.

On August 22, the seventh day after being laid, three eggs of this lot had hatched normally. The other three eggs were observed to manifest signs of the final stage of hatching.

August 23. One egg hatched on this day.

August 25. One of the remaining eggs hatched. This last larva appeared smaller at birth than the others. The embryo in the last egg died during the process of hatching.

C. Eggs kept at low temperature.—The eggs of this batch were kept in a cell slide as in the other experiments, protected from the light. The temperature controlled was slightly below outdoor temperature of 10° C. to a maximum of 20.7° C. The eggs were examined twice daily up to the thirteenth day after being laid. During this period not one of the eggs showed signs of life.

On the thirteenth day four of the eggs showed signs of hatching, two especially indicating an advanced condition. The exochorions of these two were split in several places and the form of the larva could be discerned through the semitransparent shell.

On the next day the other two eggs indicated definite signs of hatching. The two eggs which appeared as prospective larvæ on the previous day were wholly inactive and apparently dead. The two remaining eggs on this day were hopelessly shriveled beyond development.

On the fifteenth day, only one egg of the lot evinced life signs and that feebly. All of the others had collapsed and succumbed to the unfavorable environment.

In another experiment, 80 eggs of this species laid by 25 females were divided into two lots of 40 eggs each. One lot was subjected to room temperature while the other lot was placed in the basement. The temperature here was 10° C. to 15° C. Nine to thirteen days later 28 of the eggs at room temperature (18° C. to 21° C.) had hatched while the eggs at low temperature did not hatch till the nineteenth day when 22 developed into larvæ.

C. ACUTUS.

We have learned that squirrel fleas, *C. acutus*, lay eggs and produce larvæ in every season of the year. We attempted to find in what manner the extremes of temperature would influence oviposition and hatching. Two sets of experiments were conducted; one to determine the number of eggs laid and the other to determine the percentage of eggs hatched and healthy larvæ produced. Ten fertile females were placed in each of six glass beakers. Three of these vessels were provided with moistened sawdust and bran and the other three contained only the fleas; so that the eggs could be observed readily during the hatching.

Two beakers of each set were placed in the incubator, two in the refrigerator, and two kept at room temperature.

Fate of the females and eggs at different temperatures.

Temperature.	Fleas in moist sawdust and bran.	Fleas in dry beakers.
Refrigerator fleas, 5° -8° C..	Dead in twenty-nine days; no larvæ produced.	Dead in fourteen days; 6 eggs laid; none hatched.
Incubator fleas, 31°-28° C.	Dead in three days; no larvæ produced.	Dead in two days; 22 eggs laid; none hatched.
Living room temperature fleas, 15°-22° C.	Dead in thirty-six days; 68 larvæ produced.	Dead in twenty-three days; 163 eggs laid; 50 hatched.

PULEX IRRITANS.

Five of 15 eggs kept at a temperature of 11° to 15° C. hatched in fifteen to seventeen days. The same number of eggs kept as a control at room temperature hatched in seven days. Eight of the latter proved fertile.

In another experiment of a similar character 32 eggs were subjected to a temperature of 11° to 15° C. and an equal number of eggs were used as a control at room temperature. Of the eggs kept at the low temperature, 12 hatched in fourteen days, while of those kept at room temperature, 11 hatched in seven to nine days.

The larvæ upon hatching busy themselves immediately in the quest of food. They experience little difficulty in locating it, for at the time of birth a supply of food is found upon the egg shell. Here they feed from the first on the tiny blood pellicles surrounding the egg shell; this the mother furnishes when the egg is laid. When the last of the egg pellicles of blood are consumed the insatiable larvæ forage for other sustaining morsels. The dejecta of the adult flea seems to provide the desired ingredients. The young larvæ feed ravenously on the bloody deposits, apparently satisfied to pass the first few days on this unique diet. They can subsist entirely on the bloody fragments (flea fæces) when no other food is available, for a period of several days.

When food is not available the recently hatched larva nibbles its egg shell and that of its fellow (as yet unhatched). It gives evidecnce of its activity immediately after extricating itself from the egg shell, which it does head first or tail first according to the species. Whipping its way through the débris in great haste, it forages "on the run." It passes alongside an object, assays a nibble rather tentatively as if testing the quality of the food, rarely taking more than a single bite of any article.

One week after hatching the cuticle has darkened slightly but still remains quite transparent. One can readily see, with low-power microscope, as the insect feeds, how the food passes in dark masses through the mouth and along the alimentary tract, while the buccal cavity is moistened with bubbles of liquid arising from the salivary glands. The egg-opener at this stage (sixth day) has been retained, though its edge is somewhat blunted and worn.

In a batch of eggs the first larva hatched is generally, perhaps invariably, the largest. After it has hatched it chews off the bloody particles from its own shell, then nibbles on that of the others of the family prior to their hatching.

A larva of *P. irritans* was seen to emerge from the egg with head first, the tail wedged in the egg shell. The tail was freed from the shell by a relentless butting of its head against the shell where the tail was lodged. There is some evidence, from the eggs observed, that *L. cheopis* and *C. fasciatus* on the contrary emerge tail first.

The larvæ of *C. fasciatus* can live without feeding (i. e., in artificial conditions) for four to six days; and in one instance three larvæ of this species fed for nine days on an exclusive diet of flea dejecta and egg shell débris.

The color of the larva is significant in the larval cycle. At birth and for a period of three days the color is waxy white; then the

larva feeds on bloody excrement and egg pellicles and the color of the food imparts a reddish-brown color to the internal organs of the young insect.

At the second moult this bloody color is lost, since the blood pellicles deposited by the parent have dried up and the diet is composed of dirt particles and other organic débris. We have found that the intestinal tract is cleared about the third week. At this time the color of the cuticle turns to grayish and then to buff, which color is uniform until the third moult prior to spinning of the cocoon.

GENERAL METAMORPHOSES.

The long life of our native parasites seems to be anticipated at the very incubation. Eggs of the various species are laid at all times. Oviposition has been observed during every month. The broods appear to be constant and irregular. The incubation stage of our eastern forms, taken from the observations of Pergande, consumes two to four days ordinarily. Two days is given as the incubation period for the *cheopis* in India. Our experience with this species indicates a duration varying from nine to thirteen days at room temperature. When eggs of this species were subjected to identical conditions in which wild rats were caged in the laboratory basement (at a temperature of 10° to 15° C.) the length of the egg stage was nineteen days.

Larval life is cited by American and English authorities as a minimum of eight days and a maximum of twenty-four days. The larval stage of our California fleas under laboratory conditions is rarely less than twenty-eight days, often thirty days, and sometimes longer.

Pergande found that the pupal or cocoon stage of the *Pulex irritans* varied from five to seven days in the summer months at Washington. Other authors working with this species give twelve days during the summer months. We have observed several instances of cocoon life during the warm days of September. Here a maximum of thirty days was spent in this stage. During the colder season this flea remained in the cocoon as long as thirty-four days.

The entire life cycle, including adult life, is given by several authors as four to six weeks. The advisory committee on plague investigations in India gives as the time necessary for the completion of the cycle of development (in the case of *L. cheopis*)—that is, from the egg to the imago—as twenty-one to twenty-two days.

The following is given as a type of the life cycle observed in *C. acutus*. This species was kept under observation from the moment the egg was laid by its parent. An hour after the egg was laid it was placed in a small vial with a little sawdust, sand, wheat grains,

and squirrel hair. It was allowed to develop in this environment until matured.

Stage of development.	Date.	Age (days).
	1909.	
Egg laid	May 4	
Hatched	May 12	8
Molted (second stage)	May 18	14
Molted (third stage)	May 28	24
Cocoon	June 9	36
Adult	July 10	67
Alive (never having been fed)	Aug. 11	99

The following table gives the length of time required for the various stages of several species (under different climatic conditions). The data from India were taken from the reports of the English advisory committee on plague in India. The data from Australia were gleaned from the "Reports of plague in Australia, 1903," by J. Burnett Ham; those from Europe were compiled from accounts of Raillet and others; those of the Atlantic coast from Howard and Pergande, and the Pacific coast data are the result of our experience in the San Francisco Bay region of California.

The cycle of development in different countries.

[Compiled from accounts of various authors.]

Country and species of flea.	Egg.	Larva.	Pupa.	Complete generation.
India: L. cheopis	2 days	1 week	7 to 14 days	21 to 22 days.
Australia: P. irritans	6 days	12 days	14 days	4 to 6 weeks.
Europe:				
P. irritans	4 to 6 days	11 days	12 days	4 to 6 weeks.
Ct. canis	2 weeks	12 days	10 to 16 days	5 to 6 weeks.
United States:				
Atlantic coast—				
P. irritans / Ct. canis	2 to 4 days	8 to 24 days	5 to 7 days	2 to 4 weeks.
Pacific coast—				
P. irritans	7 to 9 days	28 to 32 days	30 to 34 days	9 to 10 weeks.
L. cheopis	9 to 13 days	32 to 34 days	25 to 30 days	9 to 11 weeks.
C. acutus	7 to 8 days	26 to 28 days	24 to 27 days	8 to 9 weeks.
C. fasciatus	5 to 6 days	24 to 27 days	24 to 26 days	7 to 8 weeks.

LONGEVITY OF THE CALIFORNIA FLEA.

The English workers in India ascertain the length of time which adult fleas live on rats as forty-one days. The longest life of this species on an exclusive diet of human blood was observed to be twenty-seven days. We have managed to keep this species (*cheopis*) alive for a period of thirty-six days by feeding on man. The length of life without feeding was also noted. This period varied in the experiments of the English commission according to the environment

in which the fleas were maintained. In the absence of liquid food supply, fleas could live in bran for six days, in gunny sacking, a similar time, and in sand with moist cow dung for thirteen days. From our observations we have found the majority of fleas of all species to die in five days unless a moist medium was provided.

As we have stated above rat and squirrel fleas may be kept alive for a considerable time when moisture is provided in some form. It is interesting to note that fleas which have never tasted animal food, having emerged from the cocoon and kept under the same conditions in similar material as fleas which were fed for several days and then starved, will prove longer lived. A number of specimens of *Ceratophyllus acutus* removed from a ground squirrel and kept in moistened wheat grains and sawdust lived for twenty-six days. An equal number of fleas of the same species bred from cocoons in the laboratory were kept without a host in a similar medium. One male lived thirty-eight days and a female lived for sixty-five days.

In a series of experiments in which fleas taken from healthy rats and squirrels were fed daily on the arm of a man, we attempted to determine the maximum longevity of these parasites. The fleas were placed individually in open test tubes and at feeding time the tubes were inverted over the arm of one of the laboratory attendants. Fleas were thus applied daily for a period of from five to fifteen minutes, but only the actual feeding time was recorded. The average was about five minutes.

A *Lamopsylla cheopis* was fed for thirty-five days, escaping on the thirty-sixth day. One *C. acutus* died after fifty-eight days and another one at the end of fifty-one days. The common brown rat flea, *C. fasciatus*, proved to be the most persistent feeder of them all. Unfortunately one of them was permitted to escape after feeding on its induced host for sixty-three days. Another of the group suffered no ill effects from its enforced diet of human blood for ninety-eight days; and the sole survivor had been nourished by its foster host since its removal from the rodent host for a period of five months. The experiment was discontinued, but the parasite survived a week longer in a starved condition.

LONGEVITY RELATIVE TO SEX.

We have noted the relative longevity of the sexes under the conditions of experiments, in which fleas were fed on human blood alone. The experiments with each species were conducted during the same month and under similar conditions.

We shall take for consideration the two species common to rats, *L. cheopis* and *C. fasciatus*, and the predominant squirrel flea, *C. acutus*. Four tests with *C. fasciatus* give the following data:

C. FASCIATUS.

Six males of this species averaged eight and one-half days, the maximum life being seventeen days. Fifteen females gave an average of thirty-two and four-fifth days with a maximum of one hundred and sixty days. The two unfed controls (male) of this series lived for three days, and the two control females lived for five days.

L. CHEOPIS.

Two tests with a total of seven males of this species gave an average of ten and one-seventh days, and a maximum period of fifteen days.

Three females lived twenty-eight and one-third days as an average and a maximum period of forty-nine days.

The two unfed male controls lived five days, and in four females the average was five and one-half days and the maximum was seven days.

C. ACUTUS.

One test with this species furnishes the following data: Three males averaged eleven days, and in fact all died on the eleventh day.

Five females averaged fifteen and one-fifth days. The longest life was fifty-three days.

The unfed males averaged three days and showed a maximum of four days. The female control lived five days.

The greatest length of life of a male of any species is seen to be seventeen days; and the maximum period for a female was one hundred and sixty days, somewhat over five months.

In these tests for longevity it should be borne in mind that no attempt is made to arrive at the initial age of the fleas, but the time is reckoned from the day of removal from the host.

An effort was made to determine the length of adult life of one species, *C. acutus*, by feeding the insect newly emerged from the cocoon. Ten specimens, emerging within a few hours of each other, were fed on the same day on human blood. One specimen, a female, lived for sixty-four days, at which time the experiment was discontinued. The unfed controls, as well as the specimens given a human blood diet, were kept in ordinary test tubes at room temperature. The activities of these fleas may have been influenced by changes in the temperature and the absence of moisture in the tubes.

THE REACTION OF FLEAS TO LIGHT.

In the matter of the reaction of fleas to light, no opportunity has been presented for the obtaining of accurate data with special apparatus. The observations were made in a cursory way under nearly natural conditions, and no attempt was made to distinguish the kinetic from the photic influences.

THE LARVA.

The larva is positively heliotropic up to the stage of the initial molt. The more advanced sluggish larvæ are repelled by the light. This is seen when examining the flea-breeding cages; a slight stirring of the nesting material attracts to the surface the tiny very active larvæ. If the older larvæ are desired, it is found necessary to nearly invert the container. Prior to the final molt, when the larva is in readiness to pupate, it can be seen almost invariably along the edges at the bottom of the box, where the greatest number of cocoons are brought to view.

THE ADULT FLEA.

Rodent fleas are negatively heliotropic (repelled by light) to a very striking degree. The first impulse seems to be to seek protection from the light. This is seen in combing a squirrel or rat recently killed: the fleas will retreat constantly to the underside, always in the direction away from the light. When shaken off, they return to the shadow of the host; in numerous instances when the animal was dead for a period ranging from twenty-four to fifty hours, the fleas when shaken off would seek the host and bury themselves under the hairs away from the light.

When a number of live squirrel fleas and rat fleas were placed in an open test tube, and held horizontally, with the operator's thumb covering the mouth of the vial and the bottom held against the window, the fleas crowded toward the open mouth in the direction of the thumb, bounding away from the window in an excited manner. When the tube was reversed with the open mouth toward the window, it was found unnecessary to plug the mouth of the tube, as the fleas did not attempt to jump out when given an opportunity to do so. Even when placed within a half inch of the open end of the tube with the head of the insect turned toward the light, the fleas reversed and jumped toward the closed end of the tube. This was repeated by tilting the mouth downward to offer an easier exit through the open mouth, but even this inducement did not influence the fleas, which invariably sought the closed end of the tube in the shadow. This was tried with squirrel and rat fleas, as well as with human fleas, and always with the same result.

NOTES ON THE FEEDING PROCESS.

There is a remarkable degree of variation in the feeding habits of the different species of fleas. We have not attempted, except in a superficial way, to study the idiosyncrasies of the rodent fleas in regard to the biting of their normal hosts. We have, however, quite thoroughly observed the manner of biting under experimental con-

ditions with man as a host. Without taking into account the attraction or repulsion which may be exerted toward man as a host, we shall consider the more striking features of the biting of the parasites. We find that the species do not all attack with equal avidity. *Pulex irritans*, the normal parasite of man, is insatiable in its blood craving. It differs in its relation to man in being more fastidious in its feeding than the rodent fleas. Although its bite is painful, it does not voluntarily feed in one spot for any great length of time. The *Pulex irritans* differs from all other species (hundreds of specimens of which were tested on human hosts) in that it squirts blood per anum during the act of biting.

The *L. cheopis* and *C. fasciatus*, the normal rat parasites, are found to bite man with equal readiness and will live about the same length of time when fed on human blood. Their biting is well defined and effective, but not nearly so painful as that of *Pulex irritans*, nor so prolonged as that of the common squirrel flea, *Ceratophyllus acutus*. A specimen of *C. acutus* when starved for several days has been observed to feed on man uninterruptedly for a period of nearly one hour (fifty-nine minutes) at one insertion of its proboscis.

The bite of the *Ctenopsyllus musculi*, the blind flea of the mouse, is the feeblest we have had inflicted from any flea tested. The short, weak, piercing organs of this species make a puncture, which is scarcely perceptible. In observing the length of time this flea bites it is necessary to depend as a guide on the distention of the abdomen with blood rather than the prick of the mandibles. The *musculi* seems not able to adapt itself as an induced parasite of man; from a few experiments it appears to live not longer than five days on a human host.

The *Ceratophyllus acutus*, which proves a very ready parasite of man, makes its attack even under natural conditions as well as those of an experiment. Our data compiled from reports and collections of squirrel hunters shows that this species, as well as the other common squirrel flea, *Hoplopsyllus anomalus* Baker, will bite man when exposed to their attacks.

The unusually long rostrum in the *C. acutus* is presumed to be the cause of its prolonged feeding at one insertion of the mouth parts. A correlation between length of sucking organs and the time required for feeding has been noted in other insects.

The following is the description of the method of feeding observed in *C. acutus*. This method is typical.

The flea when permitted to walk freely on the arm selects in a few minutes a suitable hairy space where it ceases abruptly in its locomotion, takes a firm hold with the tarsi, projects its proboscis, and prepares to puncture the skin.

A puncture is drilled by the pricking epipharynx, the sawtooth mandibles supplementing the movement by lacerating the cavity formed. The two organs of the rostrum work alternately, the middle piece boring, while the two lateral elements execute a sawing movement. The mandibles, owing to their basal attachments, are, as is expressed by the advisory committee on plague investigations in India (Journal of Hygiene, vol. 6, No. 4, p. 499), "capable of independent action, sliding up and down but maintaining their relative positions and preserving the lumen of the aspiratory channel." The labium doubles back, the V-shaped groove of this organ guiding the mandibles on either side.

The action of the proboscis is executed with a forward movement of the head and a lateral and downward thrust of the entire body. As the mouth parts are sharply inserted, the abdomen rises simultaneously. The hind and middle legs are elevated, resembling oars. The forelegs are doubled under the thorax, the tibia and tarsi resting firmly on the epidermis serve as a support for the body during feeding. The maxillary palpi are retracted beneath the head and thorax. The labium continues to bend, at first acting as a sheath for the sawing mandibles, and as these are more deeply inserted, it bends beneath the head with the elasticity of a bow, forcing the mandibles into the wound until the maxillæ are embedded in the skin of the victim. When the proboscis is fully inserted, the abdomen ceases for a time its lateral swinging.

The acute pain of biting is first felt when the mandibles have not quite penetrated and subsequently during each distinct movement of the abdomen. The swinging of the abdomen gradually ceases as it becomes filled with blood. The sting of the biting becomes gradually duller and less sensitive as feeding progresses. The movements of the elevated abdomen grow noticeably feebler as the downward thrusts of the springy bow-like labium become less frequent.

As the feeding process advances one can discern through the translucent walls of the abdomen a constant flow of blood, caudally from the pharynx, accompanied by a peristaltic movement.

The end of the meal is signified in an abrupt manner. The flea shakes its entire body, and gradually withdraws its proboscis by lowering the abdomen and legs and violently twisting the head.

When starved for several days the feeding of the rat fleas is conducted in a rather vigorous manner. As soon as the proboscis is buried to the full length the abdomen is raised and there ensues a gradual lateral swaying motion, increasing the altitude of the raised end of the abdomen until it assumes the perpendicular. The flea is observed at this point to gain a better foothold by advancing the fore tarsi, and then, gradually doubling back the abdomen, it turns with extreme agility, nearly touching with its dorsal side the skin of

the hand upon which it is feeding. Meanwhile, the hungry parasite feeds ravenously.

It is interesting to note the peculiar nervous action which the rodent fleas exhibit immediately when the feeding process is completed or when disturbed during the biting. Even while the rostrum is inserted to the fullest the parasite shakes its head spasmodically; in a twinkling the mouth is withdrawn and the flea hops away.

POSSIBLE VITAL CONSIDERATIONS INVOLVED IN FEEDING HABITS.

We have previously noted that rodent fleas can live in a starved condition, away from the host, during a period of three to ten days, when kept in dry test tubes—as long as twenty-eight days when a suitable moist medium is furnished. The long periods of starvation appear not to affect the vitality of the parasite to such an extent that the ability to feed is impaired. We have recorded instances in which a specimen of the squirrel flea, *H. anomalus*, starved for sixteen days, and several specimens of *C. acutus* starved for twenty-seven days had sufficient energy to feed ten to fifteen minutes when an arm was placed in the breeding jar. These facts led us, with others, to accredit in a measure the claims of older authorities on plague, who contended before the flea theory was recognized that clothes and baggage of an infected community harbored the germs of plague, which remained viable during long periods of time. Modern writers have called our attention to the plausibility of infection in clothes and baggage, due to the agency of fleas, which may be transported from infected communities. Bannerman (Journal of Hygiene, 1906, vol. 6, pp. 189–199), writing on the possibilty of the spread of infection by means of clothes, brings forth several instances in which conveyance of infection by clothes seemed the most likely means of introduction of plague in some villages in India. Plague-infected rat fleas secreted in the clothes en route are held responsible for the transmission. Bannerman cites an instance of plague transmission through clothing where as much as ten days elapsed in the tranportation of clothing removed from a plague-infected victim and worn by a relative of the deceased, who in turn contracted plague.

THE PROLIFICNESS OF C. ACUTUS.

HOW MANY YOUNG WILL ONE FEMALE PRODUCE?

The "Reports of the advisory committee on plague investigations in India" (Journal of Hygiene, 1908, vol. 8, No. 2, p. 243), cites an experiment in the reproduction of *L. cheopis*. In this instance 200 fleas of both sexes were placed on rats as the host. The experiment was conducted by placing 40 fleas on each of 5 rats. The eggs of

these fleas were removed at intervals and permitted to develop to maturity.

At the end of the third week the fleas produced	86
At the end of the fourth week the fleas produced	329
At the end of the fifth week the fleas produced	527
At the end of the sixth week the fleas produced	456
Total production in six weeks	1,398

The following experiments were conducted in San Francisco for the purpose of ascertaining the reproductive powers in a single flea:

The fleas provided in this experiment were of the species *C. acutus* kept under observation during the cocoon stage. They were collected within twenty-four hours of the emergence from their cocoons and not permitted to feed. We may state in this connection that ordinarily the newly emerged insect does not feed for as much as three days, at which time it has quite dried and the chitin begins to harden. The new insects were examined with a lens and segregated as to sex. They were distributed among the hosts (white rats) in the following numbers:

	Males.	Females.
Series 1.		
Cage 1	3	1
Cage 2	9	3
Cage 3	15	5
Series 2.		
Cage 4	6	[a]6

[a] Collected *in copulo* while on the live squirrel.

These parasites were placed in small flea-proof boxes with a layer of wheat grains and sawdust and a small white rat for a host. The rat was fed on wheat and fresh carrots and enough fresh sawdust was added from time to time to absorb the moisture which accumulated.

In series 1 the fleas were distributed in the proportion of 3 males to every female, to insure the fertility of the latter, as we have learned from numerous observations that the male assumes the passive role in the copulatory act.

In series 2 the fleas used in cage 4 were taken from a squirrel by means of a camel's hair brush dipped in ether. Six pairs of these fleas while stupefied were placed in the cage with the white rat. This series was used to check the results of series 1, since we were not able to determine at what age breeding takes place.

During the time the rats were used to feed the parasites the bedding of the cage was not removed, so that the natural conditions for flea

development were maintained. A layer of dry sawdust was sprinkled in the cages every week.

Five weeks elapsed before the hosts were removed from the cages. They were carefully combed, and the fleas obtained from this source, as well as those collected from the bedding, were placed in glass vials to ascertain if more eggs would be deposited. After two weeks no eggs were found in the vials, therefore, it was assumed that oviposition was completed. The flea breeding boxes were placed in a flea-proof closet of the laboratory and examined occasionally.

The fleas as they emerged were anæsthetized and examined microscopically to insure against "hold overs" having escaped the vigilance of the observer at the time the host was removed. The appearance of the stomach contents in the absence of mammalian blood, and the "new" appearance of the parasite were certain criteria that the flea had recently emerged. In all the cages it was interesting to note that males made their appearance one to three days prior to the females.

The fleas first to emerge were observed on February 16, 1910, in cage 4, where the parent fleas were placed on the host December 8, 1909, ten weeks previously. The last fleas to emerge from this box were collected within three days of the appearance of the first.

The total number of fleas produced from the six females was 183, an average of 30.5 per female.

In the other series of three cages the fleas emerged, as anticipated, at a later date than the progeny of those found mating. The first to appear in this series was a male, the product of cage 2. The date of emergence was March 23, 1910, after a period of fifteen weeks.

Emergence occurred in this series during a period of ten days. The boxes were observed ten days longer before they were discarded. The following enumerates the results of the reproduction of these fleas:

	Females used.	Number produced.
Cage 1	1	9
Cage 2	3	60
Cage 3	5	114
Total fleas produced		183
Average per female		20.3

In this test to determine the number of fleas produced by a single female we must not overlook certain salient factors.

There is doubtless a seasonal variation in the different broods due to environmental changes. Although this species, *C. acutus*, lays eggs at all seasons of the year, it is probable that the number deposited and the percentage hatched fluctuate markedly. Other considerations

would include the change of host from squirrel to white rat and the fact that animals in cages, especially white rats, clean themselves of parasites. It is perhaps needless to state that a study of this nature would entail observations covering a long period of time in order to obtain accurate data.

SEASONAL DISTRIBUTION OF RAT FLEAS.

Breeding experiments with rat fleas of the species *C. fasciatus*, *L. cheopis*, *Ct. musculi* convince us that propagation takes place in all seasons. Flea-breeding cages supplied with a rat for a host furnished new fleas in every month of the year 1909. Fleas removed from these rats at all times laid eggs which produced larvæ, and despite the fact that these cages were drained of fleas constantly for experimental purposes, to the extent of 1,500 *C. fasciatus* from one cage, at the end of one year there was no apparent diminution in numbers of parasites in the adult form. The entomologist of the advisory committee on plague investigations in India (Journal of Hygiene, 1908, vol. 8, No. 2, p. 242) found that in Bombay the *L. cheopis* bred in every month for one year, February, 1906, to February, 1907. However, flea breeding in the month of June was found to be not so vigorous as during the rest of the year.

In relation to the prevalence of rat fleas in California due to seasonal variations we shall consider mainly the ecological phases. The occurrence of epizootic plague in various countries has prompted several investigators to carry on extensive enumerations of fleas taken from rats. The workers in India and in Australia have established beyond doubt a direct correlation in occurrence of rat plague to rat fleas.

The tremendous "clean up" during the recent epizootic of plague in San Francisco has probably influenced the flea count of rats trapped in that city. The destruction of innumerable rat harbors and rat nests during the sanitary campaign interferred materially with the normal conditions of parasitism in these rodents. The nest of rodents generally is the ideal collecting ground for fleas, and in adapting themselves to the environmental changes induced by the federal sanitarians thousands of rats were driven from their normal nesting places into the sewers where they were trapped and brought to the laboratory and examined by us for their parasites. The search for fleas on these animals proved not very fruitful, as we soon learned that the sewer produced anything but optimum conditions for flea life. The live rats examined were in the main taken from city sewers, consequently our records prove unserviceable for the present discussion.

The conditions found in the towns of Berkeley and Oakland, present for our purposes better normal faunal interrelations. We shall omit the consideration of epidemiology by presenting data gleaned during nonepizootic times, a period embracing an enumeration of twelve months.

These counts were made and reported to us by Mr. August Venzke, laboratory assistant at the Oakland branch of the plague laboratory. We are greatly indebted to this careful painstaking collector for the data presented.

In order to prevent the fleas from leaving the rats while the latter were being transported in bright daylight or sunlight, the method of procedure selected by the last Indian plague commission was adopted by Acting Asst. Surg. William B. Wherry, then in charge of the Oakland branch laboratory, as follows:

When a rat catcher approaches a trap containing rats he places rats, traps, and all in a heavy canvas bag, which is tied securely and sent to the laboratory where the rats and their fleas are chloroformed simultaneously and a careful search is made on the body of the rat and through the contents of the bag for fleas.

The table following shows the results obtained.

Month.	Rats trapped.	Fleas collected.	Rats without fleas.	Average fleas per rat.	Monthly mean temperature.
1909.					° F.
January	38	93	12	2.44	51.0
February	360	1,274	65	3.53	50.8
March	409	1,458	43	3.56	51.9
April	531	1,127	192	2.12	58.0
May	604	1,201	159	1.98	58.4
June	739	2,534	183	3.42	61.8
July	617	1,497	141	2.42	63.8
August	447	1,232	79	2.75	61.8
September	317	903	55	2.82	62.8
October	321	1,213	42	3.77	58.8
November	206	811	56	2.77	53.3
December	237	328	69	1.38	47.9
Total	4,916	13,671	1,096	32.96	
Yearly average fleas per rat				2.74	

Judging from the table of the flea census for the twelve months we find but a slight variation in the general average of fleas per rat. Here we find a relatively even balance preserved in flea infestation throughout the year. The average number of fleas to each rat per month is not materially influenced, according to our table, by the monthly mean temperature.

The variation in the individual infestation of the rats enumerated in the monthly census shows all gradations of 99 fleas to one rat, 89 fleas to 2 rats, 37 fleas to 3 rats, and 1 flea among 5 rats. During the height of the plague epizootic, September, 1907, Acting Assistant Surgeon Wherry collected as many as 342 fleas from a single rat.

THE USE OF ANIMALS FOR FLEA TRAPPING.

Due to the successful experiments of the British Indian commission in the use of guinea pigs for trapping fleas (*L. cheopis*) in plague-infected houses, it was thought that these animals would prove of service in collecting fleas in houses in San Francisco.

Four experiments with guinea pigs were conducted by us in houses heavily infested with fleas. In every instance the animals were kept overnight in the basement of the house. Some of them were permitted to roam at large, others were exposed in wire cages to the attacks of the fleas. The "controls" used in these experiments were "tanglefoot" paper and the bare legs of the investigators.

In the first experiment 179 fleas were removed from the legs of two attendants who entered the house long enough to distribute the animals. The "tanglefoot" paper left overnight in the region of the cages trapped 232 fleas. The 6 guinea pigs yielded 2 fleas, *P. irritans*. Three other experiments of a similar character were tried in houses during the year 1909. A summary of the results of the four experiments is shown in the following table:

Animals used.	Fleas trapped by animals.	Fleas caught in tanglefoot.	Fleas taken from men.
	P. irritans.	*P. irritans.*	*P. irritans.*
6 guinea pigs	2	232	179
4 guinea pigs	4	428	60
6 guinea pigs	8	451	222
6 guinea pigs	3	120	12
22 guinea pigs	17	1,231	473

In another experiment guinea pigs were used to trap fleas in a house where dogs were responsible for the invasion of the parasites. The fleas taken from the 12 adhesive papers numbered 1,192, of which 836 were *P. irritans* and 356 were *Ct. canis*. Four guinea pigs yielded 9 specimens of *Ct. canis* and 2 specimens of *P. irritans*.

Results of much the same character have been reported by the advisory committee on plague investigations in India (Journal of Hygiene, 1908, vol. 8, No. 2, p. 218). Here the use of guinea pigs for trapping cat and dog fleas did not prove fruitful. In these experiments where man and guinea pig were simultaneously used to trap the fleas, it appears "that man is selected in preference to the guinea pig by the cat flea."

Observations in houses on *P. irritans* cited by the same authors (p. 247) proved the guinea pig a poor flea trapper. They state:

> This flea, *P. irritans*, is very select in the choice of its host. We have found it almost exclusively on man. On one or two occasions only, has a human flea been noted on a rat or on a guinea pig.

In one experiment two guinea pigs were used in a house which had been vacant for several days; here the animals failed to attract any of the fleas, while a man acted as an admirable trap. Experiments with the rat fleas, *L. cheopis*, on the other hand, demonstrated that the guinea pig is an ideal flea trap for this species. We may say here that the rat flea, *L. cheopis*, has never been encountered in our experience in houses in San Francisco.

The guinea pig, we have found, makes an admirable trap for squirrel fleas, *C. acutus*. In one instance a guinea pig was placed five minutes in the laboratory basement, which was infested with squirrel fleas, and attracted 13 fleas. When this animal was left overnight in the same quarters it had trapped 138 fleas of the species *C. acutus*. We have used guinea pigs and white rats in parallel experiments to trap rat fleas, *C. fasciatus*, and squirrel fleas, *C. acutus*. The animals were kept in wire-cage traps and exposed idividually to the attacks of the fleas in large breeding boxes. Each animal was left in the flea box for ten minutes, removed, and anæsthetized. The fleas were collected and returned to the box. After a guinea pig was tested in this manner, a white rat, one-half hour later, was given the same treatment. Five tests were made individually with 5 guinea pigs and 5 white rats. The two species of fleas, *C. fasciatus* and *C. acutus*, were trapped from distinct boxes.

Fleas trapped by the animals.

	Specimens.
Five guinea pigs:	
C. fasciatus	88
C. acutus	385
Five white rats:	
C. fasciatus	220
C. acutus	28

We may conclude from these experiments that the guinea pig is selected in preference to the white rat by the squirrel flea, *C. acutus*, and the white rat is selected in preference to the guinea pig by the rat flea, *C. fasciatus*.

SOME PHASES OF THE OCCURRENCE OF PULEX IRRITANS.

In taking the census of the flea population of thousands of rats the observer is impressed by the correlation of the prevalence of the human flea in dwellings and on rats. It appears that the increase or decrease of *Pulex irritans* is synchronously parallel in human and rodent infestation.

The sanitarian can thus often keep informed of the nature of the rodent's haunts by the presence or absence of certain of these parasites, *P. irritans*. A sewer rat, for instance, is never found ordinarily to harbor human fleas; and during house-flea infestation the rat

trapped in the dwelling is quite certain to be accompanied by *P. irritans*. In July, 1908, during a severe invasion of *Pulex irritans* in certain quarters of San Francisco, the infestation on rats from this district amounted to 61.5 per cent of the *irritans*. In the month of March of the same year 1,469 rat fleas were examined, of which only 7 specimens were *Pulex irritans*. The census of the previous month revealed a total of 2 fleas of this species taken in a collection of 1,524 rat fleas. As far as we could determine, these two months yielded a very small number of human fleas in dwellings of San Francisco.

We may say without doubt that *Pulex irritans* is preeminently the flea found in houses and upon man on the Pacific coast.

At this juncture we may call attention to the difference in infestation of the houses of the eastern United States and that of those of California. Howard, writing of house fleas in Circular No. 108, February, 1909, United States Bureau of Entomology, states:

> Judging from the specimens of fleas sent to the Bureau of Entomology of recent years with complaints of houses being infested by them, the human flea (*Pulex irritans*) is not the species most likely to occur in great numbers in dwelling houses in this country, but rather the common cosmopolitan flea of the dog and cat (*Ctenocephalus canis*). This holds especially for the eastern United States.

Wherry (Journal of American Medical Association, 1908, vol. 51, No. 6, p. 495), writing on "Fleas on rodents and men on the Pacific coast," records the fleas collected during the period from October, 1907, to February, 1908. These parasites were gathered in the "worst infected district" of a plague focus from human beings, beds, and bedding. He cites the following species:

	Specimens.
Ceratophyllus sp	2
Ctenocephalus canis	2
Pulex irritans	334

Doane in Notes on Fleas Collected on Rat and Human Hosts in San Francisco enumerates the species and numbers of fleas collected from human hosts and houses during February to June, 1908. In February fleas were taken from human hosts or on their clothing in the plague laboratory and hospitals and in refugee camps. There were 220 specimens, all of *Pulex irritans*. From March to June the total number of fleas identified from human hosts was 696 specimens; 693 of these were *Pulex irritans*, while 3 specimens were *C. fasciatus*.

Thus we find not a single specimen of *Ctenocephalus canis* in a collection of 916 fleas, covering a period of five months, taken from scores of men and houses.

Another list of fleas taken in California from man is recorded by McCoy and Mitzmain in Public Health Reports (1909, vol. 24, No.

29, p. 1018). Here we have enumerated a collection of fleas embracing a period of one year from 29 individuals.

Pulex irritans, 337 specimens; *Ctenocephalus felis*, 1 specimen; *C. canis*, 1 specimen, and *Ceratophyllus acutus*, 3 specimens.

We may conclude from these results that the human flea, *Pulex irritans*, is the normal parasite of man on the Pacific coast.

HIBERNATION OF PULEX IRRITANS.

In which stage and at what season does the human flea hibernate?

Observations were made by the writer during the winter months of 1907, 1908, and 1909 to determine in what stage of development the human flea, *P. irritans*, hibernates in California. During the course of this study he inspected 14 badly flea-infested dwellings, from which were collected the adult fleas, dust of the floors, and soil immediately under the houses. The parasites and the dirt collected were placed in separate vials in the laboratory, where they were carefully observed.

Note was taken of the egg laying and longevity under artificial conditions. It was a matter of interest to observe that the females which at warmer seasons lay five to eight eggs at a time, now deposited, at the most, two eggs, and the majority did not oviposit. In all cases the eggs laid failed to hatch. Another feature of interest in the winter forms appeared in the greater resistance to starvation and unfavorable conditions. Under these circumstances this species does not ordinarily survive beyond a period of five days. The wintering females averaged eight days, some surviving twelve days.

The organic matter, which was collected at the same time and in the same surroundings as the adult fleas enjoyed, was inspected from time to time for the development of flea life. These samples were kept for thirty to forty days to provide for the later development of any cocoons which might have been present. In no instance did we find the larval form of this flea; in only one instance did we find an adult flea which either developed from a cocoon or had been overlooked at the time the material was collected from the dwelling. Several instances of the egg stage were noted. These did not hatch, however, but shriveled after a few days.

We may add in this connection that temperature experiments with *P. irritans* have borne out the facts adduced from nature. Gravid females of this species were collected during the summer months and placed in shell vials in a refrigerator maintained at a temperature between 8° C. and 10.2° C. Under these conditions eggs were laid, but failed to hatch.

It is quite apparent therefore that the house flea, *Pulex irritans*, hibernates most probably in the winter in the adult stage.

As we have demonstrated, this species, in comparison to the rat and squirrel fleas, does not resist starvation for any considerable time, certainly not during the full period of the hibernating stage, which may extend to two months or ten weeks. By this we mean the period when man is free from their attacks. The question arises upon what host does this insect feed in the absence of man, the normal host. That is to say, what animal, if any, is the temporary host during the "interrupted" hibernation period. The host which naturally presents itself is that nearest to man and most accessible to the parasites. The dog is probably the host selected.

We have collected fleas from dogs during the winter months in the bay cities of Berkeley, Oakland, and San Francisco. In December, 1907, and January, 1908, when fleas in houses were rarely found, we collected fleas from 10 house dogs in Berkeley. The number actually collected was 456 fleas, although the animals harbored probably four times this number. The fleas identified were:

	Specimens.
Ctenocephalus canis	322
Pulex irritans	134

Twenty-nine per cent of the total catch were human fleas.

The writer in investigating the cause for the immunity of certain people to the biting of fleas has made extensive inquiries among his intimate associates as to the data of attacks by these parasites. From January 5, 1910, to March 30, 1910, reports of the flea infestation among persons ordinarily susceptible were very few. During this period we inspected three houses, the basements of which were swarming with fleas. The history of the infestation of the dwellings was similar in all of them. In each case the basement, the region of greatest infestation, was the harborage of two to three dogs which were more or less confined to these quarters. Six of these dogs were examined for fleas, when it was seen that 40–55 per cent of the parasites of each dog were *Pulex irritans*. The domiciles of these hosts were examined and many fleas collected. From the worst infested of the houses we collected overnight on adhesive paper 1,192 fleas which were identified as follows:

	Specimens.
Ctenocephalus canis	356
Pulex irritans	836

Here we find 70 per cent of the fleas collected, with the dog as an occasional host, to be the house flea, *Pulex irritans*.

In a report of the fleas collected during the plague campaign in California (Public Health Reports, 1909, vol. 24, No. 29, p. 1019), Passed Asst. Surg. G. W. McCoy and the present writer recorded

the following parasites taken from four dogs during Februray and March, 1909:

Ct. canis.		P. irritans.		Ct. felis.		C. acutus.	
M.	F.	M.	F.	M.	F.	M.	F.
10	44	8	17	0	1	0	1
54		25		1		1	

In this enumeration 30 per cent of the fleas found were *Pulex irritans.*

During the warmer seasons we have collected fleas from dogs in San Francisco Bay regions and find a great preponderance of *Ct. canis.* Doane reports in Canadian Entomologist (August, 1908, p. 303), the identification of fleas from a Dachshund pup. These parasites were sent to him by former Acting Assistant Surgeon Wherry in the latter part of April, 1909. There were 477 specimens taken from the dog; without one exception the fleas were of the species *Ctenocephalus canis.*

With this somewhat meager data, we can not establish with faultless accuracy that the human flea is tided over the hibernation stage to a limited extent on the body of the dog, but we wish to suggest this as a plausible solution.

We have not had an opportunity to give the attention to the domestic cat in the same measure as the dog has been investigated, but we doubt not that the feline having similar environments would be influenced in this matter of parasitism to perhaps the same degree. We may state, however, in this connection, that an examination of a dozen cats during a period of two years revealed the presence of few human fleas.

THE CAUSE FOR THE UNEXPECTED APPEARANCE OF HOUSE FLEAS.

It was long held in the popular mind that in some quarters fleas were generated spontaneously. We may say that this impression from the layman's view point seems justified when it is known that the reason for the sudden appearance of fleas in houses is often obscure.

In Circular No. 13, United States Bureau of Entomology, Howard states that a housekeeper shutting up her house in June need not be surprised to find the establishment overrun with fleas when she opens it up again in September or October.

In Parasitology 1, page 10, March, 1908, appears a statement that observers have recorded that the flea propagates in deserted dwellings,

the adult insect not requiring food, at least for some time, to enable it to reproduce its species. An observation of a similar character is recorded by Euting, 1896, Reise in Inner-Arabien, page 11.

In common with these investigators we can cite a number of definite instances of this apparent spontaneity in house-flea infestation. However, this phenomenon is too well established for further needless substantiation.

We have, however, gathered experimental evidence as a control to our general observations. On numerous occasions we have accompanied the sanitary inspector on his rounds of many filth-infested refugee cottages, whence dust and débris were removed from cracks in the floors. This material was taken to the laboratory, placed in convenient flasks, and left undisturbed in a dark corner.

When first examined, no life was in evidence, some inert shells of the adult flea and moulted larval skins were observed. In one instance, when the flask was inspected, five weeks later, two live fleas appeared followed in ten days by fifteen additional active imagoes.

At other times the floor dust when gathered from a dwelling was supplied with many larvæ in various stages of development. These transformed into adult fleas when kept in the laboratory for a period of four to six weeks.

No doubt if the houses from which flea material were collected should be vacated and left undisturbed for certain periods, the swarm of fleas produced would indeed give the unfortunate housekeeper an impression of spontaneous generation.

○

H
21
78
39

TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

PUBLIC HEALTH BULLETIN No. 39
SEPTEMBER, 1910

STUDIES UPON LEPROSY

IX. MOSQUITOES IN RELATION TO THE TRANSMISSION OF LEPROSY

X. FLIES IN RELATION TO THE TRANSMISSION OF LEPROSY

BY

DONALD H. CURRIE

PASSED ASSISTANT SURGEON AND DIRECTOR LEPROSY INVESTIGATION STATION

XI. HEREDITY VERSUS ENVIRONMENT IN LEPROSY

BY

HARRY T. HOLLMAN

ACTING ASSISTANT SURGEON LEPROSY INVESTIGATION STATION
PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE

Investigations made in accordance with the Act of Congress approved March 3, 1905

Library
School of Hygiene
University of Toronto
LIBRARY
SEP 5 196
UNIVERSITY OF TORONTO

WASHINGTON
GOVERNMENT PRINTING OFFICE
1910

TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

PUBLIC HEALTH BULLETIN No. 39

SEPTEMBER, 1910

STUDIES UPON LEPROSY

IX. MOSQUITOES IN RELATION TO THE TRANSMISSION OF LEPROSY

X. FLIES IN RELATION TO THE TRANSMISSION OF LEPROSY

BY

DONALD H. CURRIE

PASSED ASSISTANT SURGEON AND DIRECTOR LEPROSY INVESTIGATION STATION

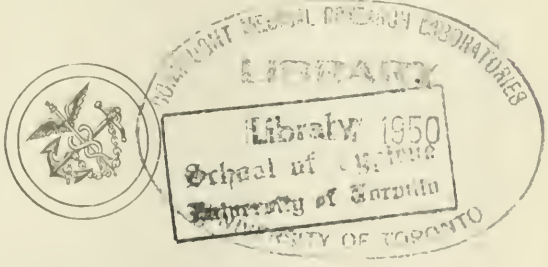
XI. HEREDITY VERSUS ENVIRONMENT IN LEPROSY

BY

HARRY T. HOLLMAN

ACTING ASSISTANT SURGEON LEPROSY INVESTIGATION STATION
PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE

Investigations made in accordance with
the Act of Congress approved
March 3, 1905



WASHINGTON
GOVERNMENT PRINTING OFFICE
1910

MOSQUITOES IN RELATION TO THE TRANSMISSION OF LEPROSY.

By Donald H. Currie,
Passed Assistant Surgeon and Director, Leprosy Investigation Station.

PART I.—INTRODUCTION.

In the nodular form of leprosy when fully developed we have a disease characterized by the appearance, in successive crops, of lesions of the skin varying in size from that of medium-sized papules to large nodules, and, by the coalescing of such lesions, massive and diffuse areas of infiltration.

These lesions are most apt to be located on the exposed surfaces, and especially on the face.

When an investigator punctures such a lesion and exerts pressure on the surrounding tissue, the mixture of blood and lymph thus obtained is usually, practically always, rich in lepra bacilli.

In the mosquito we have an insect that has a wide range of distribution and is often abundant. This insect feeds by puncturing the skin wherever uncovered, but more often on the face, and fills itself with blood. It later bites another person, who often mashes the blood-filled insect and thus smears the blood contained in the insect upon his own skin; this act is followed by rubbing and scratching the site of the puncture.

Thus we see that the mechanism is complete for the occasional transfer of a leper's *blood* to the skin of a healthy person, and by scratching and its accompanying trauma such blood is sometimes rubbed into hair follicles or even into the lymphatics. Whether the blood that the insect has imbibed from a leper contains the bacilli, and whether such bacilli if imbibed remain in the insect a reasonable length of time, are questions the answer to which determine whether the mechanism for the transfer of the bacillus of leprosy from afflicted to healthy persons is or is not complete.

To determine this is the object of this investigation. It must be borne in mind, however, that even if it can be shown that the bacilli are thus transferred there still remains a doubt as to whether this constitutes a means of infection. We shall not deal with that point; in our present state of knowledge only human inoculations could

determine it. A hasty review of the literature upon that phase of the subject of transmission shows some forty-odd cases of alleged human inoculations, mostly on or under the skin, with but a single, doubtful, case of infection following (Arning's case), the other inoculated persons not developing the disease.

The only phase of the subject we are dealing with here is, Do mosquitoes contain lepra bacilli when allowed to feed, under natural conditions, upon the skin of lepers presenting many lepra nodules? If they do contain the bacilli, then the mechanism for the transfer of the bacilli is complete, and the insect *may* be a factor in the transmission of the disease. If it does not contain them, then this insect can be excluded as a factor in the transmission of this disease.

In considering this subject we are aware of the fact that no amount of negative evidence absolutely answers the question, "May it not sometimes occur?" but from the incidence of the disease at certain times, and in certain places, we know that some well-defined and frequently acting mechanism or mechanisms exist for its transfer, and we seek to discover if this insect is one of these. The eradication of infectious diseases must rest upon the discovery of the *usual* means of transmission, and rare or accidental means are of no practical importance.

From the above it would appear, upon theoretical grounds, that the transfer of the bacilli is probable; a disease characterized by bacilli-filled lesions on exposed surfaces, together with the well-known habits of this insect of imbibing blood from the skin of such exposed surfaces, would easily explain the fact that this insect fell under suspicion as a means of transmission. But it must be remembered, also, that although the investigator finds bacilli in the blood obtained from puncturing a lepra nodule, this insect employs a similar but not identical method of blood abstraction.

REVIEW OF THE LITERATURE.

Leloir,[a] 1886, thought mosquitoes possible agents in the transmission of leprosy.

Ashmead,[b] 1890, believes lepra would be found to have an intermediate agent, either a fish or an insect.

Arning,[c] 1890, states that he has examined "hundreds" of mosquitoes caught in the bars over lepers, but he has never found the lepra bacillus in the insects.

[a] Traité pratique et théorique de la lèpre, quoted by Marchoux and Bourret in Lepra, vol. 8, p. 120.

[b] Lepra in Japan. Ist ein Zwischenwirt bei der Verbreitung beteiligt? Journ. of Cut. and Gen.-Urin. Diseases. Referred to in Baumgarten's Jahresbericht, vol. 6, p. 265.

[c] Lepra mit besonderer Berücksichtigung der Uebertragung durch Heredität oder Contagion. Referred to in Baumgarten's Jahresbericht, vol. 6, p. 247.

The same author the following year [a] mentions that lepra and mosquitoes entered the Hawaiian Islands at nearly the same time.

Of this latter statement we desire to further refer to Ashburton Thompson's article "Leprosy in Hawaii" (1898), in which he shows that the claim that leprosy entered these islands at the time referred to rests on very uncertain data, and may or may not be true.

Joly,[b] 1898, states that Sabrazes believed that leprosy might be conveyed by numerous small inoculation from insects.

Sommer,[c] 1898, expressed the belief that mosquitoes act as active agents in the spread of leprosy in warm countries. In referring to this Nuttall comments, "this is very unlikely."

Hutchinson,[d] 1899, gives six reasons against the probability of leprosy being spread by mosquitoes.

Scott,[e] 1900, believes that mosquitoes acted as transmitters of leprosy in the Hawaiian Islands.

Chantemesse,[f] 1901, favors the mosquito hypothesis as explaining the spread of leprosy.

Joly,[g] 1901, does not regard mosquitoes as transmitters, as Europeans are often bitten and rarely develop the disease.

Hallopeau,[h] 1901, favors the mosquito hypothesis as explaining the transmission of leprosy.

Blanchard,[i] 1901, states that leprous countries are also mosquito countries.

Tonkin,[j] 1902, believes that mosquitoes, by causing scratching, favor infection with leprosy in the Sudan where lepers sell soiled clothing.

---

[a] Archiv für Dermatol. und Syph., No. 1, and referred to in Lepra, vol. 8, p. 120.

[b] Importance du rôle des insectes dans la transmission des maladies infectieuses et parasitaires. Du formol comme insecticide. Referred to by Nuttal in Johns Hopkins Hospital Reports, vol. 8, p. 33.

[c] Leprosy in the Argentine Republic. Sem. Med. of June 23, 1898, and referred to by the Journal of American Medical Association, September 10, 1898; by Nuttall in Johns Hopkins Hospital Reports, vol. 8, p. 34; by Marchoux and Bourret in Lepra, vol. 8, p. 121.

[d] Is leprosy spread by mosquitoes, etc.? Archives of Surgery, vol. 10, No. 37, p. 59. Referred to in Baumgarten's Jahresbericht, vol. 15, p. 391.

[e] Contagiousness of leprosy. British Medical Journal, September 29, 1900. Reviewed in Lepra, vol. 8, p. 177, and referred to by Marchoux and Bourret in the latter publication, vol. 8, p. 120.

[f] Bull. de l'Acad. de Méd., July, 1901. Referred to by Marchoux and Bourret in Lepra, vol. 8, p. 120.

[g] Lèpre à Madagascar. Arch. de Méd., Nov., 1901. Referred to by Lepra, vol. 3, p. 57.

[h] Leçons cliniques de l'hôpital. Bull. de l'Acad., July, 1901. Referred to by Marchoux and Bourret in Lepra, vol. 8, p. 120.

[i] Bull. de l'Acad. de Méd., July 30, 1901, and Archive de parasitologie, 1901. Referred to by Marchoux and Bourret in Lepra, vol. 8, p. 120.

[j] Some general and etiological details concerning leprosy in the Sudan. Royal Med. and Chir. Soc. of London. Referred to in Lepra, vol. 3, and Baumgarten's Jahresbericht, No. 18.

Noc,[a] 1902, does not believe in direct contagion, but considers the bite of the mosquito a possible means of spread.

The same author,[b] 1904, believes mosquitoes helped to spread leprosy in the Loyalty Islands. On one of these islands there were few mosquitoes and the disease spread very little.

Jeanselme,[c] 1906, states that the spread of leprosy in New Caledonia is probably increased by mosquitoes.

Goodhue,[d] 1906, claims that after many failures he and Father Joseph discovered lepra bacilli in a female culex.

Black,[e] 1906, states that it has not yet been determined whether insects played any part in the spread of leprosy in Cape Colony.

Smit,[f] 1906, rejects the idea that insects are factors in the spread of leprosy.

Miser,[g] 1906, refers to Joly's and Sabrazès's opinions in the matter. (Vide supra.)

Hallopeau,[h] 1906, states he believes that mosquitoes are factors in the spread of leprosy.

Brinckerhoff,[i] 1908, states "the fact that the female culex defecates at the time of biting makes it possible that the insect may act as carrier of leper bacilli from lepers to well persons. The data now available does not permit of a positive statement that the mosquito functions in the transmission of leprosy, and the probabilities are against such a conclusion." He established the fact that the mosquito defecates at the time of biting, and in the same paper reports having seen acid-fast bacilli in a preparation stated to have been made from a mosquito that had fed on a leper.

---

[a] Fonctionnement du laboratoire de bactériologie de Nouméa (N.-Calédonie). Annales d'hygiène et de méd. colon. Referred to in Baumgarten's Jahresbericht, vol. 19, p. 355; also Lepra, vol. 4, p. 208.

[b] La lèpre aux Iles Loyalty. Annales d'hygiène et de méd. colon., vol. 7, No. 1, p. 5, also referred to in Lepra, vol. 4, p. 186, and in Baumgarten's Jahresbericht, vol. 20, p. 507.

[c] In a report of Hallopeau and de Lepinay, entitled, "Nouvelle poussée de nodules érythémateux chez une lépreuse." Soc. franç. de dermatol. et de syphil., vol. 17, p. 378, and referred to in Baumgarten's Jahresbericht, vol. 22, p. 327.

[d] Boston Med. and Surg. Journ., 1906; American Medicine, Oct., 1907; Journal of Trop. Med., September 15, 1906; Indian Medical Gazette, 1906; Lancet, May 12, 1906; Lepra, 1907, p. 64. Also commented on by Ehlers in 1909 in Report of Danish-French Commission, presented at Bergen.

[e] Remarks on leprosy in Cape Colony. Centralbl. f. Pathol. u. pathol. Anat., Bd. 17, p. 476.

[f] Leprosy in Argentine. Arch. f. Dermatol. und Syph., vol. 91, p. 389.

[g] Prophylaxie des maladies exotiques. Baumgarten's Jahresbericht, vol. 22, p. 343.

[h] Brissaud-Gilbert. Maladies exotiques, p. 280. Referred in Baumgarten's Jahresbericht, vol. 22, p. 537.

[i] A note upon the possibility of the mosquito acting in the transmission of leprosy. Bull. of the Leprosy Investigation Station, Kalawao, Molokai, U. S. P. H. & M. H. Service.

Matias Duque,[a] 1909, states that Doctor Agramonte had shown him a preparation made from a mosquito which showed lepra bacilli, and that he had observed them in others since, but he does not believe these insects play any part in its spread.

MacLeod,[b] 1909, states "so far the microbe has not been found in the mosquito."

Ehlers [c] states that they (the Danish-French commission) obtained negative results in their search for lepra bacilli in these insects even when the latter were allowed to feed upon leprous nodules. The explanation of this fact is not clear, but he believes that it may be due to the mosquito puncturing a small vessel and obtaining lymph-free blood, while the investigator when he punctures a nodule obtains a mixture of blood and lymph.

Bourret,[d] 1909, states that:

First, he captured from lepers' homes three *Stegomyia fasciata* (*callopus*); examined one after one hour, one after two hours, and one after three hours, but in none did he note the lepra bacillus.

Second, he allowed two *Culex*, two *Anopheles*, and two *Stegomyia* to bite over a leprous nodule and examined the insects, some after fifteen minutes and the rest after an hour with negative results.

Third, he repeated this experiment on a case of nerve leprosy with negative results.

SUMMARY OF THE LITERATURE.

Aside from those authors who hold views on this subject apparently based on theoretical grounds, we also have the following:

Those who searched in vain for the bacillus of leprosy in the mosquito are—

Arning, who has examined "hundreds" of these insects caught under the bars of lepers.

Ehlers (Danish-French commission), that actually placed the captive mosquitoes over nodules and allowed them to feed.

Bourret, who examined three mosquitoes from lepers' houses and six mosquitoes that had fed on leprous nodules.

Of those that have seen acid-fast bacilli in mosquitoes that were believed to have fed upon lepers we have—

Goodhue, Brinckerhoff, and Matias Duque. The two former in a single insect; the latter's statement does not contain information as to the number of insects he has observed containing the bacilli.

PRELIMINARY REMARKS.

The following data have been obtained by work performed at the laboratory for the investigation of incipient cases of leprosy at Hono-

[a] Sanidad y Beneficencia. Habana, June, 1909, p. 384.

[b] Lancet, August 21, 1909, and read at Bergen, August 16, 1909.

[c] Transmissibilité de la lèpre par les insectes suceurs de sang, read at Bergen.

[d] Quelques recherches sur la lèpre. Lépra, vol. 8, p. 128.

lulu, being a branch of the United States leprosy investigation station at Kalawao, Molokai.

The portion of Honolulu where this branch laboratory is situated is infested with the *Culex cubensis* throughout the year, but in our experience in the autumn and winter the *Stegomyia callopus* are rare in this exact locality, while the *Stegomyia scutellaris* has not been observed there by me.

These three varieties are the only mosquitoes known to exist on these islands. The above facts account for all the mosquitoes examined being of one species. During these experiments we succeeded in securing only three individual specimens of *Stegomyia callopus;* these we had intended to utilize by having them bite lepers' nodules, but administrative duties made it necessary for us to go to Molokai, and for this reason the three were used for another purpose, to be described later.

Arriving at Molokai station, we discovered that owing to the cooler temperature and high winds of this time of the year no mosquitoes were procurable, so decided to publish the data already obtained and later to report upon the contemplated work of having caged mosquitoes bite a large number of leprous nodules.

The necessity of obtaining the *Stegomyia* in this latter work is due to the fact (observed here by Brinckerhoff) that this *Culex* will not bite when confined to a small space, and in order to force the insect to feed upon a nodule confinement to a large test-tube or wide-mouth bottle is necessary.

PRELIMINARY WORK.

Our first object was to ascertain whether mosquitoes, when captured in a locality in which there was no possibility of their having fed upon lepers, contained acid-fast bacilli of any kind in their intestinal tracts; also whether they showed anatomical structures which might from shape and staining reaction be mistaken for acid-fast bacilli.

With this object in view we obtained 21 specimens of *Culex cubensis* from a noninfected district, some of these having fed upon the healthy occupants of the house in which they were captured.

The abdomen was cut from the thorax and opened and the intestines and their contents crushed and smeared upon a glass slide. This preparation was then air-dried, stained from three to five minutes in Ziehl-Nielson carbo-fuchsin, washed in water, treated with 1 per cent nitric acid in 70 per cent alcohol for ten seconds, washed again in water, dried, and examined with the 1:12 oil immersion lens (Zeiss).

This method of examination was employed in all of the insects mentioned in this publication and so will not be referred to again.

The examination of the 21 insects mentioned showed no acid-fast bacilli or bacteria of any kind.

Numerous fibrils, varying in shape, size, and appearance (broken bits of the scales that cover parts of the insect) were noted as holding their stain against acid alcohol. These structures for the most part could be differentiated at a glance upon size and shape alone, but occasionally it was observed that small broken fragments required careful observation before passing them by; however, we saw no structure which a reasonably experienced person should mistake for an acid-fast bacillus.

Hoping to secure a method that would deprive these structures of their acid fastness without robbing the lepra bacillus of its color, and thus rule out even this very slight source of error, we took smears of human and rat lepra bacilli and smeared mosquitoes over them (these insects not being included in the 21 mentioned), after which these slides were dried, fixed, and stained.

These were treated with many stains and mordants, with the results that while an occasional slide was secured in which the bacillus was brilliantly red while the structures mentioned had as different a color as blue black, the rule was failure, these structures being nearly as acid-fast as the bacilli themselves. The most practical point learned was to avoid staining the slide longer than absolutely required (three minutes), as the bacilli acquired the stain somewhat quicker than the scales did.

While not "preliminary work" in one sense, as it was the last work performed, the experiment about to be described properly belongs under this designation.

We sought for an explanation of the statements of Ehlers and Bourret that mosquitoes drawing blood from a bacilli-filled nodule did not contain the bacilli in their blood-filled intestines. If this observation were correct, then there seemed to be but two possible explanations:

First. That the bacilli were not contained in the fluid that the mosquito drew, even though the investigators, in attempting to imitate the mosquito, drew the fluid from the same nodule.

Second. That the bacilli are rapidly destroyed in the intestinal tract of the mosquito.

Any filtration process could be excluded, both from the anatomy of the mosquito and from the fact that blood corpuscles passed into the insects' intestinal tracts without difficulty.

While to us the second supposition seemed quite improbable, it was considered advisable, if only to exclude it, to make the following test:

The three *Stegomyia* referred to were placed in separate cages and given a fluid composed of rabbit serum, sugar, and water, and containing in suspension a grass bacillus, which in size and acid fastness closely resembled the bacillus of leprosy. Of these insects two

refused to feed, the third lit near the margin of the fluid and twice dipped the end of its proboscis into it, exhibiting the corkscrew motions of its abdomen that indicated that the insect was feeding.

One hour after this act the insect was killed with chloroform and removed from its cage, its body was washed and the abdomen separated from its thorax and its abdominal contents smeared upon a slide and stained by the method above described. Examination showed a considerable number of typical acid-fast bacilli in its intestinal contents, 45 being counted in seven fields; no other bacteria were demonstrated by the methylene blue counter stain. The experiment is to be repeated with lepra bacilli, and will appear in a future publication.

Search for the Bacillus of Leprosy in Blood-Filled Mosquitoes that Probably Had Fed upon Lepers.

We secured 34 blood-filled specimens of *Culex cubensis* caught on a screen door at the entrance to the Kalihi receiving station for lepers. In this compound there were about 20 lepers, showing nerve, tubercular, and mixed forms of the disease. Several healthy persons also resided in this compound. The screen door mentioned was a few yards from the patients' sleeping rooms, which were not protected by screening. These insects had probably fed upon some of these lepers, but on which cases, and therefore which type of the disease, was not ascertainable. The examination was made only because the insects were at hand and a possibility of securing positive results existed.

The results, however, were entirely negative, and therefore, owing to the uncertainty of the source of the blood, they are of only slight value.

Search for *Bacillus lepræ* in Blood-Filled *Culex cubensis* That Had Fed upon Lepers.

These insects were captured early each morning from the rooms of lepers; some of these rooms were occupied by one and some by two lepers; the condition of the occupant or occupants of each room is described below. The letters given the rooms are our own, and are for purposes of designation and convenience.

The probability of any of these insects having fed upon nonleprous persons is too slight to be considered. The probability of any appreciable percentage of them having fed upon lepers, not occupants of the room and therefore not those described, is very small.

Rooms and Brief Description of the Cases Occupying Each.

Room A: Occupied by two lepers, one a purely nerve case, the other showing general leprous infiltration over a large portion of the face.

Room B: Occupied by one leper. Large nodules about ears and a few small ones on face.

Room C: Occupied by one leper, showing numerous small papules and nodules scattered over face.

Room D: Occupied by one leper, showing diffuse infiltration of lobule of one ear and numerous small nodules scattered over the face.

Room E: Occupied by two lepers, both advanced cases, showing nodules and infiltration over a large part of their faces.

Room F: Occupied by two lepers, one showing small nodules scattered over face and some diffuse infiltration, the other case showing extensive leprous infiltration and nodules scattered over a large part of the face.

Room G: Occupied by one case, showing diffuse infiltration about malar regions and lobules of the ears.

Room H: Occupied by one case, showing infiltration of lobules of both ears, no other lesions on face.

Room I: Occupied by one case, showing nodules scattered over face, with inner one-third of face showing general infiltration.

The following table is self-explanatory; all the preparations and examinations were made by the method previously described. All the insects referred to in this table were blood-filled female specimens of *Culex cubensis*. The smear was made from thirty minutes to three hours after the capture of the insect.

Designation of room.	Number of cases.	Type of cases.	Number of mosquitoes procured from this room and examined.	Result of the examination of these insects.
A	1	Nerve case	108	No acid-fast bacilli found.
	1	Tubercular		
B	1	do	68	Do.
C	1	do	71	Do.
D	1	do	100	Do.
E	2	do	17	Do.
F	2	do	95	Do.
G	1	do	2	Do.
H	1	do	14	Do.
I	1	do	18	Do.

Tubercular cases exposed to mosquitoes	11
Blood-filled mosquitoes examined	493
Acid-fast bacilli found	0

DISCUSSION.

The failure to find bacilli in the insects may be supposed to be due to one or more of the following causes:

First. The cases were not suitable ones for the test.

Second. By chance the insect failed to bite over a nodule.

Third. The insect when at liberty purposely avoids nodules and chooses the areas of healthy skin.

Fourth. For some reason (as previously mentioned by Ehlers and Bourret) the mosquito does not take in bacilli when it feeds from the blood of leprous nodules.

Fifth. That the bacilli are soon destroyed in a mosquito's stomach.

As to the first of these reasons, the cases were all of the nodular type except one, and were average cases of the middle period of the disease; i. e., most of them would neither be called "very early" nor "advanced" cases.

As to the second point it is believed that the number of the insects examined excludes, on the face of it, such a supposition.

As to the third it appears quite improbable, as we know nothing analogous to it in the mosquito's feeding habits; this point is to be investigated in our future work.

As to the fourth supposition, this appears to most easily explain our results, and is borne out by the observations of two previous investigators; this is also to be investigated.

As to the fifth, the single experiment was so strikingly positive that this supposition is considered most improbable, but will be repeated with lepra bacilli.

To return to the first supposition, this raises a somewhat different question, which, so far as we know, no one has attempted to investigate, and in fact its investigation would be next to impossible; that is, whether a mosquito feeding upon a leper at the time of the existence of a bacillæmia (during the last stages of the disease) would not at times imbibe the organism.

None of our cases were advanced enough to lead one to suspect that a bacillæmia existed. That such a condition does occur at times is fully testified to, but the relatively small number of bacilli present even in such cases is also shown by the persons that have sought for it in vain. These two facts, the rarity of leprous bacillæmia and the relatively small number of bacilli present per unit of blood, are sufficient to exclude, on mathematical grounds, such an occurrence as being of epidemiological importance.

CONCLUSIONS.

Mosquitoes feeding, under natural conditions, upon cases of nodular leprosy so rarely, if ever, imbibe the lepra bacillus that we can exclude them as one of the ordinary means of transferrence of this bacillus from lepers to the skin of healthy persons. This insect is therefore not of epidemiological importance in this disease.

I desire to acknowledge the aid furnished me by Messrs. Fulloway and Terry, United States Department of Agriculture, in informing me as to the species name of the *Culex* found in Honolulu.

PART II.—A FURTHER NOTE ON THE FAILURE TO FIND LEPRA BACILLI IN MOSQUITOES THAT HAD FED UPON LEPERS.

In the previous paper[a] we published the negative results obtained by us from the examination of 493 female mosquitoes that had fed under natural conditions on cases of nodular leprosy. In that paper we stated that we expected to pursue these studies further as soon as female specimens of *Stegomyia callopus* could be obtained.

The examination referred to of the 493 mosquitoes demonstrated only that these insects, when feeding under natural conditions, did not contain lepra bacilli in their digestive tracts after such feeding. The present work was undertaken in hope of ascertaining the reason or reasons underlying the above fact.

In the first article we stated that one of the following reasons must account for the absence of the bacilli in these insects after such feeding:

First. That the cases of leprosy were not suitable ones for the test

Second. That by chance the insects had failed to bite over a leprous nodule.

Third. That the insect, when at liberty, purposely avoids nodules, and chooses the area of healthy skin.

Fourth. That for some reason, as previously mentioned by Ehlers and Bourret, the mosquito does not take in the bacilli when feeding from a leprous nodule.

Fifth. That the bacilli are soon destroyed in the mosquito's stomach.

The first of these we excluded by a description of the cases, which were of a type that afforded the mosquitoes every opportunity to obtain bacilli; the question of chance in the second supposition could be excluded on the face of it by the large number of insects examined. The remaining three we hoped to clear up in the experiments to be described in these notes.

LITERATURE.

In our first article on this subject we reviewed the literature, so shall not consider it here, except to state that in the former article we omitted to mention the negative results of the British commission to India for the study of leprosy (1890–1893).[b] In their experiments with a number of mosquitoes that had fed on lepers they report failure to find bacilli in thirty smears that they examined;

[a] Mosquitoes in relation to the transmission of leprosy, Part I.

[b] Report reviewed by Lancet, 1893, vol. I, p. 1153, and Baumgarten's Jahresbericht, vol. 9, p. 273.

also an article by Ch. Nicolas, entitled "Moustiques et Lepre,"[a] in which the author, by reasoning from epidemiological data, believes the mosquito to play a part in the spread of leprosy.

We desire also to invite attention to the reference made to Ehlers's report on the results of the Danish-French commission (1909). The results of our experiments coincide with theirs, in so far as mosquitoes are concerned, and the conclusions drawn as to the probable explanation of the failure of this insect to imbibe the bacilli are identical with theirs, although we arrived at these conclusions in a somewhat different manner.

EXPERIMENTS.

A. To determine whether or not mosquitoes, when imbibing a fluid containing lepra bacilli, will not only take in these bacilli, but will retain them, morphologically unchanged, in its digestive tracts for a reasonable length of time.

Experiment 1.—Placed 14 of these insects in a jar and gave them a suspension of lepra bacilli in water. Withdrew all other fluid to force them to imbibe of this suspension. At the end of twenty-four hours we killed the insects with chloroform. There was no way of determining which of these insects, if any, had imbibed this fluid except by the presence of lepra bacilli in their digestive tracts.

Smeared the abdominal contents of these insects on glass slides, fixed them by heat, stained them for three minutes in Carbo-Fuchsin (Ziehl-Nielson), decolorized for ten seconds in 1 per cent nitric acid in 70 per cent alcohol, counter stained with Loeffler's Methylene Blue, and examined these stained smears under the oil immersion lens.

9 of these insects showed no bacteria of any kind in their intestinal tracts.
5 of these insects showed a large number of lepra bacilli in their intestinal tracts.

These organisms were typical and unchanged in appearance, even to the occurrence in characteristic clumps, the largest of these clumps containing 10 lepra bacilli. Many oil immersion fields showed 20 or more lepra bacilli, although the smears were of only average thickness.

Experiment 2.—Placed 6 of these insects in a cage containing a similar lepra bacilli suspension in water.

Twenty-four hours later we killed these insects with chloroform, smeared their intestinal contents on glass slides, and stained them by the method described in the last experiment:

4 of these insects showed no bacteria of any kind in their intestinal contents.
2 of these insects showed numerous typical lepra bacilli in their intestinal contents.

[a] Bull. Soc. pathol. exot., 1908, p. 493, quoted by Lepra, vol. 8, fasc. 4, p. 243.

B. Experiments having a twofold object:[a]

First. To ascertain whether the female *Stegomyia callopus* shows any preference between the normal skin and the skin over leprous nodules.

Second. To ascertain whether mosquitoes which had actually fed over a leprous nodule did or did not contain lepra bacilli in their digestive tracts.

Experiment 1.—Placed 5 female specimens of *Stegomyia callopus* in a large-mouth bottle and placed the bottle over a leprous nodule; 3 insects bit the adjacent normal skin, while 2 bit over the nodule. Two hours later all 5 insects were killed with chloroform and their abdominal contents stained and examined. None of these insects showed any acid-fast bacilli in their digestive tracts. Of course it was not to be expected that the 3 that bit the normal skin would contain such bacilli, but they were all examined as a matter of routine.

Experiment 2.—Placed 3 female specimens of *Stegomyia callopus* over a leprous nodule, by the same method described above. They all lit on the normal skin, but refused to feed, probably due to the fact that the atmospheric temperature was low, and the room in which the experiment was performed was not well lighted.

Experiment 3.—Placed 7 female specimens of *Stegomyia callopus* over a leprous nodule; 5 refused to bite, 1 bit the normal skin, and 1 bit over the leprous nodule. Examination of the 2 insects that had fed failed to show any acid-fast bacilli.

Experiment 4.—Placed 9 female specimens of *Stegomyia callopus* over a leprous nodule; 3 bit the normal skin and 6 refused to bite at all. Examination of the 3 insects which had bitten the normal skin was, as would be expected, negative.

Experiment 5.—Placed 2 female specimens of *Stegomyia callopus* over a leprous nodule; both bit the normal skin.

Experiment 6.—Placed 1 female specimen of *Stegomyia callopus* over a leprous nodule; insect bit the normal skin.

Experiment 7.—Placed 4 female specimens of *Stegomyia callopus* over a leprous nodule; 3 bit the normal skin and 1 bit over the nodule. Examination of all 4 insects showed no acid-fast bacilli in their intestinal tracts.

Experiment 8.—Placed 4 female specimens of *Stegomyia callopus* over a leprous nodule; 2 bit the nodule and 2 bit the normal skin. Examination of the 4 insects gave negative results as to acid-fast bacilli.

---

[a] In all these experiments the leprous nodules were rather small, not occupying more than one-third of the total skin area surrounded by the mouth of the bottle, so that on an average the mathematical chances of the insect biting a nodule was as 1 is to 2 of its biting the normal skin.

Experiment 9.—Placed 1 female specimen of *Stegomyia callopus* over a leprous nodule; it bit the skin over the nodule. Examination of the insect failed to show any acid-fast bacilli in its intestinal tract.

Experiment 10.—Placed 1 female specimen of *Stegomyia callopus* over a leprous nodule. In biting, the insect chose the adjoining normal skin instead of the nodule. After a two hours' interval we smeared the abdominal contents of this insect and examined it, with negative results.

It will be seen by the experiments so far recorded that of 37 mosquitoes placed over leprous nodules 7 bit the nodules, 16 bit the normal skin, and 14 refused to bite.

C. Having thus obtained sufficient data to indicate that the insect exhibited no preference for either normal skin or leprous nodule, we ceased to keep account of those insects which fed on normal skin or those insects which refused to bite at all, the recorded experiments from here on bearing only on those insects which actually punctured a well-marked leprous nodule.

Experiment 1.—Placed a number of female specimens of *Stegomyia callopus*, each in a separate bottle, over a leprous nodule; of these, 5 bit a leprous nodule. Examination of these 5 insects showed no lepra bacilli in their digestive tracts.

Experiment 2.—Placed 1 female specimen of *Stegomyia scutellaris* over a leprous nodule; the insect bit the skin over the nodule. Examination of the insect showed no lepra bacilli in its digestive tract.

Experiment 3.—Placed a number of female specimens of *Stegomyia callopus*, each in a separate bottle, over a leprous nodule; of these, 2 bit the skin over the leprous nodule. Examination of these insects showed no lepra bacilli in their digestive tracts.

Experiment 4.—Placed a number of female specimens of *Stegomyia callopus*, each in a separate bottle, over a leprous nodule; of these, 9 bit the skin over a leprous nodule. Examination of these 9 insects showed no lepra bacilli in their digestive tracts.

Experiment 5.—Placed a number of female specimens of *Stegomyia callopus*, each in a separate bottle, over a leprous nodule; of these, 7 bit the skin over a leprous nodule. Examination of these 7 insects showed no lepra bacilli in their digestive tracts.

Experiment 6.—Placed a number of female specimens of *Stegomyia callopus*, each in a separate bottle, over a leprous nodule; of these, 15 bit the skin over a leprous nodule. Examination of these 15 insects showed no lepra bacilli in their digestive tracts.

Experiment 7.—Placed a number of female specimens of *Stegomyia callopus*, each in a separate bottle, over a leprous nodule; of these, 8 bit the skin over a leprous nodule. Examination of these 8 insects showed no lepra bacilli in their digestive tracts.

Experiment 8.—Placed a number of female specimens of *Stegomyia callopus*, each in a separate bottle, over a leprous nodule; of these, 6 bit the skin over a leprous nodule. Examination of these 6 insects showed no lepra bacilli in their digestive tracts.

Experiment 9.—Placed a number of female specimens of *Stegomyia callopus*, each in a separate bottle, over a leprous nodule; of these, 7 bit the skin over a leprous nodule. Examination of these 7 insects showed no lepra bacilli in their digestive tracts.

SUMMARY.

1. Of 23 insects that were given a choice of feeding on the skin over a leprous nodule or on normal skin, 7 bit over the nodules and 16 bit the normal skin. The area covered by the nodules and the area covered by the normal skin was as 1 is to 2.
2. Total number of insects that bit leprous nodules (B plus C series), 67.
3. Number of insects that showed lepra bacilli, none.
4. Of 20 mosquitoes given an opportunity to feed on a suspension of lepra bacilli in water, 7 showed lepra bacilli and 13 showed no lepra bacilli in their digestive tracts.

DISCUSSION.

First. That if mosquitoes should imbibe any body fluid that contains lepra bacilli, they will not only take those bacilli into their digestive tracts, but will keep them there, morphologically unchanged, for several hours.

Second. That the mosquito, when it bites over a lepra-bacilli-filled nodule, does not imbibe the bacilli.

Third. That when given its choice the insect appears to show no preference for either the normal skin or a nodule.

If the first and second of these three observations are correct, then it follows that the insect, when it punctures the skin over a leprous nodule, draws only bacilli-free fluid, this alone accounting for the absence of bacilli in its body after such feeding. The investigator, in attempting to imitate the insect by puncture, invariably draws a bacilli-filled fluid. The fluid of a nodule that is rich in bacilli is the lymph. The only bacilli-free fluid that a nodule could contain is that within the vessel walls of the general circulation (capillary or small venule).

From this reasoning, based upon the data obtained in the experiments cited above, it is evident that, while it is impractical for man to obtain lymph-free blood through a puncture of the skin, this insect is able to, and does, insert its proboscis directly into a capillary or other small vessel and draws pure blood directly from the general circulation.

52478—Bull. 39—10——3

In these experiments, as in those described in the first paper, we are dealing with cases of the afebrile stage of leprosy. Our experiments, therefore, present no data as to what may occur during the febrile stage of the disease, in which bacillæmia is present. But, as stated in the foregoing paper, the stage of bacillæmia is relatively rare, and the number of bacilli per unit of blood is relatively small; even though the mosquito does imbibe bacilli in such febrile cases, it must occur too seldom to be of great epidemiological importance.

CONCLUSIONS.

First. The reason that mosquitoes that have fed on lepers do not contain the lepra bacilli is that when these insects feed they insert their proboscis directly into a blood vessel and thus obtain bacilli-free blood, unmixed with lymph.

Second. That the above-mentioned habit alone accounts for the absence of lepra bacilli in mosquitoes that have fed on lepers; the insect neither avoids biting a leprous nodule nor is its digestive tract or the contained fluids capable of altering the morphology of this bacillus in a reasonable length of time.

BIBLIOGRAPHY.

Leloir, 1886. Traité pratique et théorique de la lèpre. (Quoted by Marchoux and Bourret in Lepra, vol. 8, p. 120.)

Ashmead, 1890. Lepra in Japan. Ist ein Zwischenwirt bei der Verbreitung beteiligt? Journ. of Cut. and Gen.-Urin. Dis. (Referred to by Baumgarten's Jahresbericht, vol. 6, p. 255.)

Arning, 1890. Lepra mit besonderer Berücksichtigung der Uebertragung durch Heredität oder Contagion. (Referred to in Baumgarten's Jahresbericht, vol. 6, p. 247.)

Arning, 1891. Same title. Arch. f. Dermatol. u. Syphil., vol. 23. (Referred to in Lepra, vol. 8, p. 120.)

British Commission to India, etc., 1890–1893. Report reviewed by Lancet, 1893, vol. p. 1153; also Baumgarten's Jahresbericht, vol. 9, p. 273.

Thompson, J. A., 1898. Leprosy in Hawaii. Mitteil. u. Verhandl. d. internat. wissensch. Lepra-Konf. zu Berlin, Bd. 3, Abt. 1, p. 270; also Baumgarten's Jahresbericht, vol. 14, p. 440.

Joly, 1898. Importance du rôle des insectes dans la transmission des maladies infectieuses et parasitaires. (Referred to by Nuttall in Johns Hopkins Hospital Reports, vol. 8, p. 33.)

Sommer, 1898. Leprosy in the Argentine Republic. Semaine Médicale, June 23, 1898; also Journ. of Amer. Med. Assn., Sept. 10, 1898, and by Nuttall in Johns Hopkins Hospital Reports, vol. 8, p. 34; Marchoux and Bourret in Lepra, vol. 8, p. 121.

Hutchinson, 1899. Is leprosy spread by mosquitoes, etc.? Arch. of Surgery, vol. 10, No. 37, p. 59. (Referred to by Baumgarten's Jahresbericht, vol. 15, p. 391.)

Scott, 1900. Contagiousness of leprosy. British Med. Journ., Sept. 29, 1900. (Reviewed in Lepra, vol. 8, p. 177; also referred to by Marchoux and Bourret in Lepra, vol. 8, p. 120.)

Chantemesse, 1901. Bull. de l'Acad. de Méd., July, 1901. (Referred to by Marchoux and Bourret in Lepra, vol. 8, p. 120.)

Joly, 1901. Lèpre à Madagascar. Arch. de Méd., Nov., 1901. (Referred to by Lepra, vol. 3, p. 57.)

Hallopeau, 1901. Leçons cliniques de l'hôpital. Bull. de l'Acad., July, 1901. (Referred to by Marchoux and Bourret in Lepra, vol. 8, p. 120.)

Blanchard, 1901. Bull. de l'Acad. de Méd., July 30, 1901. (Referred to by Marchoux and Bourret in Lepra, vol. 8, p. 120.)

Tonkin, 1902. Some general and etiological details concerning leprosy in the Sudan. Roy. Med. and Chirur. Soc. of London. (Referred to in Lepra, vol. 3, and Baumgarten's Jahresbericht, vol. 18.)

Noc, 1902. Fonctionnement du laboratoire de bactériologie de Nouméa (N.-Calédonie). Ann. d'hygiène et de méd. colon. (Referred to in Baumgarten's Jahresbericht, vol. 19, p. 355; also in Lepra, vol. 4, p. 208.)

Noc, 1904. La lèpre aux îles Loyalty. Ann. d'hygiène et de méd. colon., vol. 7, No. 1, p. 5. (Referred to in Lepra, vol. 4, p. 186, and in Baumgarten's Jahresbericht, vol. 20, p. 507.)

Jeanselme, 1906. Nouvelle poussée de nodules érythémateux chez une lépreuse. Soc. franç. de Dermatol. et de Syphil., vol. 17, p. 378. (Referred to in Baumgarten's Jahresbericht, vol. 22, p. 327.)

Goodhue, 1906. Boston Med. and Surg. Journ., 1906; American Medicine, Oct., 1907; Journ. of Trop. Med. and Hygiene, Sept. 15, 1906; Indian Med. Gaz., 1906; Lancet, May 12, 1906; Lepra, 1907, p. 64; also commented on by Ehlers in 1909 in Report of the Danish-French Commission, presented at Bergen.

Black, 1906. Remarks on leprosy in Cape Colony. Centralbl. f. Path. u. pathol. Anat., Bd. 17, p. 476.

Smit, 1906. Leprosy in Argentine. Arch. f. Dermatol. u. Syphil., vol. 91, p. 389.

Miser, 1906. Prophylaxie des maladies exotiques. Baumgarten's Jahresbericht, vol. 22, p. 343.

Hallopeau, 1906. Brouardel-Gilbert: Maladies exotiques, p. 280. (Referred to by Baumgarten's Jahresbericht, vol. 22, p. 337.)

Brinckerhoff, 1908. A note upon the possibility of the mosquito acting in the transmission of leprosy. Bull. of the Leprosy Investigation Station, Kalawao, Molokai, U. S. P. H. & M. H. Service.

Duque, Matías, 1909. Sanidad y Beneficencia, Habana, June, 1909, p. 384.

McLeod, 1909. Lancet, August 21, 1909. (Read at Bergen on August 16, 1909.)

Ehlers, 1909. Transmissibilité de la lèpre par les insectes suceurs de sang. (Read at Bergen.)

Bourret, 1909. Quelques recherches sur la lèpre. Lepra, vol. 8, p. 128.

Nicolas, Ch., 1909. Moustiques et lèpre. Bull. Soc. pathol. exot., 1908, p. 493. (Quoted by Lepra, vol. 8, p. 243.)

FLIES IN RELATION TO THE TRANSMISSION OF LEPROSY.

By Donald H. Currie,
Passed Assistant Surgeon and Director, Leprosy Investigation Station.

INTRODUCTION.

Practical experience has demonstrated that in some countries leprosy can be controlled by sanitary regulations, including the isolation of the afflicted. But the fact remains that there are other localities where no amount of effort can remove the passive resistance of a large part of the population to such measures. Such resistance need consist in little more than the refusal to report their sick to the authorities in order to make the eradication of the disease next to impossible.

In its early stages, sometimes for the first few years of the disease, the symptoms are often such as to make this concealment an easy matter. In spite of this difficulty, all possible efforts should be directed to the early detection and isolation of cases, but if we could determine more exactly the means by which leprosy is spread we might expect to develop, from the knowledge thus gained, other sanitary regulations that would tend to inhibit some of the mechanisms of transfer, and thus aid the present system of isolation in the control of the disease.

See's abstract of Jeanselme's article [a] contains the following three terse sentences: "La lèpre est une maladie terrible et incurable." "La race blanche n'est pas à l'abri de la lèpre." "La lèpre se propage exclusivement par contagion." [b] These words express much—our helplessness after the disease has developed, the danger to which no people are immune, and the measures that offer the greatest chances of success, the measures looking to the prevention of contagion, direct or indirect.

[a] Lepra, Vol. III, p. 64.

[b] The author employs "contagion" in a broad sense as meaning direct or indirect contact, as is shown further on in the article by the following: " .....de toutes les professions amenant avec d'autres individus des contacts directs ou indirects (alimentation, habillement, domesticité, etc.) de l'usage des bains, hôtels, voitures publiques, etc."

But in order to carry out these measures more perfectly we must first know what constitutes indirect contact—what agencies constitute a mechanism for the transfer of the infection.

In our present state of knowledge all that we can *hope* to prove is that a certain mechanism does or does not convey the organism to the skin or mucosa of man. If we can show that a certain mechanism does not effect such transfer, we can exclude it as a factor in the spread of the disease. If, on the other hand, we can show that a certain mechanism often does convey the bacillus of leprosy from the ulcers of lepers to the skin of healthy men, we are justified, in the interests of sanitary protection, in presuming that such a mechanism is a factor in the spread of the disease. Until we have grown the bacillus on artificial media, and successfully inoculated the lower animals with such cultures, it is difficult to see how we can hope to progress beyond this stage of presumptive evidence.

LITERATURE.

The following literature has been referred to and has a direct bearing on this subject:

Report of the Leprosy Commission in India. (Lancet, May 13, 1893, p. 1153; also Baumgarten's Jahresbericht, vol. 9, p. 269.) This commission placed 10 flies on leprous ulcers, the secretions of which were rich in bacilli. The subsequent examination of these flies showed no lepra bacilli.

Corredor, 1893 (Revista Méd. de Bogotá, No. 201, and reviewed by Polakowsky in Deutsche med. Wochenschrift, No. 40, p. 646; also referred to by Nuttall (1899) in Johns Hopkins Hospital Reports, Vol. VIII), speaks of the statement of an Indian living with lepers that flies often gathered upon the ulcers of these afflicted persons, after which the insects bit him; that the site of such bites became inflamed and later ulcerated, and that from these leprosy resulted.

Nuttall, 1899, in his masterly article entitled "On the rôle of insects, arachnids, and myriapods as carriers in the spread of bacterial and parasitic diseases of man and animals" (Johns Hopkins Hospital Reports, Vol. VIII), states "it appears that Linnæus and Rolander considered that *Chlorops* (*Musca*) *lepræ* was able to cause leprosy by its bite" (Blanchard, Zool. med. II, p. 497). Nuttall also reviews this subject up to date, referring to Corredor, and quotes Joly (1898), "Importance du rôle des insectes dans la transmission des maladies infectieuses et parasitaires," as stating that Sabrazes believed that leprosy might be transferred by a large number of small inoculations from insects.

Joly, 1901 (Lèpre à Madagascar. Arch. de Méd., Nov., 1901, p. 459, and referred to in Lepra, vol. 3, p. 57), believes flies fill themselves with the secretions of lepers and carry the bacilli to others.

Tucker, 1903. A contribution to the discussion on the etiology of lepra. (Indian Lancet, 1903, vol. 24, p. 830.) Baumgarten refers to this article in Baumgarten's Jahresbericht, vol. 19, p. 335, as follows. "Believes certain fly plays a rôle in spread of leprosy, but furnishes no proof."

Stoll, 1906 (Arch. f. Dermatol. u. Syphil., vol. 91, p. 389), in writing of leprosy in the Argentine, states that he does not believe in insect transmission.

Musser, 1906, in an article entitled "Prophylaxie des maladies exotiques" (quoted in Baumgarten's Jahresbericht, vol. 22, p. 343), refers to Corredor's statement (vide supra).

Clift, 1907, in an article entitled "The intestinal origin of leprosy" (British Med. Journ., Apr. 20, 1907, p. 931; also Arch. f. Dermatol. u. Syphil., vol. 89, p. 132), raises the question of the possible transfer of lepra bacilli by means of flies from leprous ulcers to foodstuffs.

Wherry, 1908, in an article entitled "Further notes on rat leprosy and on the fate of human and rat lepra bacilli in flies" (Journ. of Inf. Diseases, vol. 5, p. 507; also U. S. P. H. and M. H. Reports, vol. 23, p. 1841), shows by experiment that several flies, including *Musca domestica*, took up large numbers of rat-lepra bacilli from the carcass of a leprous rat and deposited them all in a few days after ingestion. That the larvæ of *Calliphora vomitoria* if placed in the carcass of a leprous rat become heavily infected with lepra bacilli, but the flies produced by these larvæ seldom contain the bacilli, and then only a few. He also states that a single fly (*Musca domestica*) caught on the face of a human leper showed numerous acid-fast lepra-like bacilli.

Nash, 1909, in an article entitled "House flies" (Journ. of Hyg., vol. 9, p. 161), states "the spread of leprosy has also been attributed to biting flies."

Duque, 1909 (Sanidad y Beneficencia, Habana, June, 1909), expresses the belief that flies act as carriers of leprosy.

MacLeod, 1909 (The present state of our knowledge of the bacteriology and pathological anatomy of leprosy. Lancet, Aug. 21, 1909), states: "So far the microbe has not been found in insects suspected of disseminating the disease."

Muhlens, 1910 (Berliner Klin. Wochenschrift, Mar. 7, 1910, p. 440), refers to Wherry's work as follows: "Wherry has established the fact that lepra bacilli taken up by flies do not multiply in the latter, and are soon deposited again."

The close resemblance of the tubercle and lepra bacill make data obtained in the case of the one of a certain value in the case of the other. Therefore the following references are not out of place:

Spillmann and Haushalter, 1887 (Dissémination du bacille de la tuberculose par mouches. Compt. rend., vol. 7, p. 352), found many tubercle bacilli in the bodies and in the feces of flies that they had captured in the wards of a hospital.

Hofmann, 1888. Ueber die Verbreitung der Tuberkulose durch Stubenfliegen. Correspondenzblatt d. aertzl. Kreis- u. Bezirksvereine im Koenigreiche Sachsen, vol. 44, No. 12, p. 130, referred to by Nuttal. Found tubercle bacilli in 4 out of 6 flies caught in a ward which had been occupied by a patient suffering from tuberculosis.

Lord, 1904. Boston Med. & Surg. Journ., December 15, 1904, pp. 651–654. Publ. of Mass. Gen. Hosp., 1906, p. 118, and reviewed by Lancet, February 18, 1905, and by the Journal of American Medical Assn., March 31, 1906. Also referred to by Wherry in his article (for title, etc., vide supra), and by Howard in Bull. No. 18, U. S. Dept. of Agriculture. We have not seen the original publication, but from the several reviews and references it appears that Lord fed 30 caged flies with tuberculous sputum. During the next few days these insects deposited many tubercle bacilli (3,000 to 5,000 per speck), and by the end of the third day 26 of these 30 insects had died. Some of the references to this article mention the experiment as demonstrating that the bacilli increased in the insects' intestinal tract; that immense numbers were deposited is certain, but there is nothing in the references we have that speaks of the numbers ingested. That the tubercle bacillus should multiply greatly in three days under such conditions of temperature, etc., would seem most surprising.

Heiser, 1909 (Quarterly Report of Bureau of Health for the Philippine Islands, third quarter, 1909, p. 3), states that "the bacilli of tuberculosis may also be disseminated by flies, because the fly will feed on sputum, and the tubercle bacilli may pass through the digestive tract alive and appear in the flyspeck. Tubercle bacilli have been found alive in the excreta of flies fed with infected sputum fifteen days after the flyspecks had been deposited."

The following literature has also been referred to; it has only an indirect bearing on the subject:

Hirch, 1896. An account of two cases of coko or framboesia. Lancet, Vol. II, p. 173.

Howard, 1902 (Farmers' Bull. 155, U. S. Dept. of Agriculture), speaks of the immense productive power of the "house fly."

Nash, 1904 (Lancet, vol 1, p. 380), speaks of flies as transmitters of various epidemic summer diarrheas.

Chantemesse and Borrel, 1905 (Report of the Meeting of the Acad. of Med., of Paris, Lancet, Nov. 4, 1905, p. 1368), speak of flies in connection with the spread of cholera.

Howard, 1906 (Circular 71, U. S. Dept. of Agriculture), speaks of the several kinds of flies found about dwellings.

Newstead, 1907 (Ann. of Trop. Med. and Parasit., p. 507), speaks of the several kinds of flies found in dwellings.

Stephens and Newstead, 1908. The anatomy of the proboscis of biting flies. (Ann. of Trop. Med. and Parasit., 1907, vol. 8, p. 171.)

Glover, 1908 (Lancet, Sept. 5, 1908, p. 715), speaks of flies as carriers of infantile diarrhea.

Newstead, Dutton, and Todd, 1908 (Ann. of Trop. Med. and Parasit., vol. 1, p. 3), speak of biting flies in the Congo Free State.

Esten and Mason, 1908 (Storrs Agri. Exper. Station, Bull. 51, quoted by Howard in Bull. 78, U. S. Dept. of Agriculture), state that of 414 flies examined the average number of bacteria was over 1,000,000 per fly.

Huber, 1908 (N. Y. State Journ. of Med., Nov., 1908), states that he believes tubercle bacilli are often carried by flies.

No attempt has been made to cover the literature on the subject of flies in connection with diseases other than lepra, the above few being cited only as examples of the well-known habit of the fly of conveying certain pathogenic organisms.

The interest in and knowledge bearing on insects as disease carriers are, for the most part, quite recent; but the following is of interest as denoting that opinions on this subject were held by some at an early date: Netter (Arch. de Méd. Expérim., 1900, p. 109) quotes the following paragraph:

Imo muscas tam infirmorum tam cadaverum sicco saturates mox in alias domos vicinales transmigrantes, dum sordibus suis comestibilia inficiunt, hominibus attulisse Mercurialis refert.

Nobilis quidam in nupera peste Neapolitanea cum nescio quid ad fenestram observaret musca crabro quidam advolans naso insedit et promuscidis spiculo eidem infixo tumorem quendam effecit quo sensim crescente et intra viscera serpente veneno intra biduum haud dubie ex contagioso humore quem musca ex cadavere susceperat) confectus peste extinctus fuit.

SUMMARY OF LITERATURE.

Aside from those who apparently hold opinions based upon theoretical grounds, we have the following research work:

1. The commission to India, 1893. They placed 10 flies on the ulcers of lepers, the contents of which were rich in bacilli, but their results were negative.

2. Wherry, who examined a single fly caught from a human leper, found lepra bacilli in its intestinal contents.

In addition to the above, we have the report of Corredor; but it will be seen that the observation was not made by Corredor, but by an Indian leper, and from our information, we presume, an untrained observer. We hardly think that this observation should be given much weight.

Attention is invited in this connection to the fact that we are dealing with nonbiting flies; if it were otherwise, a little more literature could be cited, notably the Danish-French commission.

When we consider the interest recently shown in the subject of insects as disease carriers, the small amount of published researches on the subject we are dealing with is surprising. Especially is this true when we further consider that leprosy is known to occur most where social and hygienic conditions are not good. It is under such conditions as these that we are apt to find the bacilli-discharging ulcer carelessly exposed to the attacks of flies, and it is also under such surroundings that these insects are apt to be in greatest abundance.

FLIES EXPERIMENTED WITH.

Of the several species of flies found in Honolulu and Kalawao, we chose the following for our experiments: *Musca domestica*, *Sarcophaga pallinervis* (*Thomson*), *Sarcophaga barbata* (*Thomson*), *Volucella obesa* (*Fabr.*), and an undetermined species of *Lucilia*.[a]

As might be expected, we found that in and about dwellings the first-named fly is by far the more numerous; in fact, greatly exceeds in number all other kinds of dipterous insects combined. The findings in the case of this fly may, therefore, be of greater importance from a sanitary standpoint than the findings in the cases of the two kinds of *Sarcophaga* (the common blowfly in this locality). The *Lucilia* (species undetermined) is fairly common; the *Volucella obesa* could hardly be of importance, for the reason that, although occasionally found in houses, I have never seen it voluntarily light on man, and it is more often found in shaded parts of the premises, outside of the dwellings.

CONTROL WORK.

Twenty specimens of *Musca domestica* and 12 specimens each of the four other varieties were captured and killed with chloroform; their abdomens were detached and opened, the intestines removed, and their contents smeared on glass slides and fixed, after which they were stained with carbo-fuchsin (Ziehl-Nielson). They were then decolor-

[a] We are indebted to Messrs. Fullaway and Terry, entomologists, Hawaii Experimental Station, U. S. Department of Agriculture, for the classification of the above-mentioned flies.

ized in 1 per cent nitric acid in 70 per cent alcohol and counterstained with a solution of alkaline-methylene blue (Loeffler's). Examination of the smears with the oil-immersion lens showed no acid-fast bacilli. The structures mentioned in the mosquito in a previous bulletin (The Mosquito in relation to the transmission of leprosy) as holding their stain against the acid alcohol were also present in the smears of the flies, but, as in the case of the other insects, errors could hardly occur on account of these acid-fast, broken bits of scales. The above 68 insects were examined to determine whether a fly that had not been in contact with either lepers or cases of pulmonary tuberculosis contained any acid-fast bacilli which might be mistaken for the bacillus of leprosy.

PART I.—FLIES AND THE ACID-FAST BACILLUS OF MOELLER.

At the time that these experiments were first considered, circumstances were such as to make it impracticable to secure leprous material; it was therefore decided to perform certain preliminary experiments with another acid-fast bacillus, namely, the grass bacillus of Moeller,[a] and later, as soon as the leprous material should become available to us, to conduct similar experiments with Hansen's organism. By doing this we hoped to perfect a technique in the handling of the flies that would be of aid to us in the subsequent work with the lepra bacillus; then again, there was no limit to the amount of material available for the grass bacillus experiments; hence as many tests could be made as seemed advisable. It was also believed that if the results obtained with this bacillus were later duplicated by the results obtained with the lepra bacillus that this preliminary work would not be entirely lost, but would have the value of supporting evidence for the more important work to be undertaken with the bacillus of leprosy. The acid-fast bacilli which we employed in the preliminary work were grown upon ordinary nutrient agar, and were found to differ very little in size and staining reaction from the bacillus of leprosy. On an average they were a trifle shorter and more plump.

Experiments with *Sarcophaga pallinervis* (Thomson).

Experiment 1.—A guinea pig was chosen; its abdomen was shaven, washed with soap and water, and dried with a towel. By means of a pair of scissors a circular piece of its skin was clipped out, so as to form an artificial ulcer 5 millimeters in diameter, with its base formed by the fascia covering the anterior abdominal muscles. A loopful of an agar-culture of this grass bacillus was placed in this ulcer and intimately mixed with the bloody serum that had exuded into the ulcer.

[a] Moeller has isolated three distinct varieties (?) of grass bacilli; in passing through many hands the records as to which one this organism descended from has been lost.

A fly of the above species was placed in a wide-mouth bottle measuring 14½ cm. in length and 5½ cm. in circumference. The bottle was inverted, its mouth placed over the ulcer, and held in this position. The fly was not disturbed any more than was necessary in the inversion of the bottle.

The insect immediately flew to the ulcer, alighting on its edge, and fed freely from the secretions containing the grass bacilli. It then turned about and deposited a number of larvæ near the ulcer. These larvæ at once buried themselves in the secretions of the ulcer and remained there about ten minutes, when they were picked up and placed in a bottle. These larvæ were labeled "2–A" and will be referred to later.

Forty-eight hours later this insect was found dead in her cage, although food had been supplied her. Her abdomen was separated from the thorax, its intestines removed, their contents smeared on a glass slide, stained for acid-fast bacilli by the method described under "control work," and examined. This smear showed an immense number of acid-fast bacilli, identical in appearance with the grass bacillus.

Experiment 2.—Took another fly of the above species, placed it in a bottle as described in the last experiment, and placed the bottle over an artificial ulcer that had been infected with grass bacilli. The fly promptly lit on the edge of the ulcer, fed freely from its secretions, and then deposited four larvæ. The fly was placed in a clean cage and given sterile food. The larvæ were labeled "13–A" and will be referred to later.

On the following morning the adult insect was found dead; its intestinal contents were examined, and found to contain immense numbers of acid-fast bacilli.

Experiment 3.—Took another fly of the same species and placed it over another infected ulcer. The fly lit on the edge of the ulcer and fed sparingly from it, only dipping her proboscis into it for an instant, and repeating this act two or three times. She then turned around and deposited a number of maggots on the edge of the ulcer, these maggots at once burying themselves in the secretions. They were labeled "17–A," and will be referred to later. The adult insect was placed in a clean cage and fed.

On the following morning she was given another clean cage, and the specks deposited since her feeding on the ulcer were examined and an average of 6 acid-fast bacilli per oil immersion field were found in these specks.[a]

[a] This means of conveying an idea as to number has, of course, only a comparative value. We employed the Zeiss No. 1.5 with No. 4 eyepiece. On an average there were about 30 "oil immersion fields" in a flyspeck; the latter vary somewhat in size with the nature of the insect's food.

On the day following, i. e., forty-eight hours from feeding, the feces of the insect (that has been passed between the twenty-fourth and forty-eighth hour after the feeding) were again examined, but no acid-fast bacilli were found. On the same day the insect was killed with chloroform and its intestinal contents examined, but no acid-fast bacilli were found.

EXPERIMENTS WITH *Sarcophāga barbata.*

Experiment 1.—A specimen of this species was placed over a grass-bacilli infected ulcer. The insect lit on edge of ulcer and deposited six larvæ. These larvæ entered the ulcer and buried themselves in its secretions. The adult insect did not feed herself; she was removed into a clean cage, where she deposited a large number of larvæ. The larvæ which were deposited on the ulcer's edge were labeled "4–A," while those subsequently deposited in the cage were labeled "4–B," and will be referred to later. Inasmuch as the adult insect did not feed, she was destroyed.

Experiment 2.—Another specimen of this species was chosen and placed over a grass bacilli-infected ulcer. She lit near the edge of the ulcer, and fed from it for a short interval. She deposited no larvæ.

The following morning the adult insect was found dead in the cage. Examination of her intestinal contents showed several hundred acid-fast bacilli to each oil immersion field.

Experiment 3.—Another specimen was placed over an infected ulcer. She fed very little from it, but deposited a number of maggots. These maggots buried themselves in the secretions of the ulcer; after being allowed to remain there, they were placed in a clean cage and labeled "12–A," and will be referred to later. The adult insect died during the following night. Smears from its intestinal contents showed no acid-fast bacilli.

Experiment 4.—Another fly of this species was chosen and placed over a similarly infected ulcer. The insect fed, but did not deposit larvæ; it was placed in a clean cage and given food, as in the case of the previously mentioned insects. This insect invariably deposited her feces on the foodstuffs she was furnished, so that no specks for examination were obtainable. The insect died seven days after feeding. The intestinal contents showed no acid-fast bacilli.

Experiment 5.—Another fly of the same species was placed over a similarly infected ulcer. It fed, but did not deposit larvæ. The insect was given a clean cage and food.

No feces from this insect were examined for the first twenty-four hours. Those deposited two days later were examined and found to be negative. The insect was then chloroformed, but no acid-fast bacilli were discovered in its intestinal contents.

EXPERIMENTS WITH *Lucilia* (SPECIES UNDETERMINED).

Experiment 1.—A specimen of this species was chosen and placed over an ulcer infected with grass bacilli. It at once lit upon the ulcer, walked over its surface, and fed freely from its secretions.

The following morning the insect was found dead; examination showed a moderate number of acid-fast bacilli in its intestinal contents.

Experiment 2.—Another fly of this species was placed over an ulcer in the same manner as in the previous experiment. The insect lit by ulcer and walked across its surface, but it was not possible to determine whether it had fed or not.

The following morning the insect was found dead. Examination of its intestinal contents showed no acid-fast bacilli.

EXPERIMENTS WITH *Volucella obesa* (FABR.).

Experiment 1.—Placed a specimen of this species of insect over an infected ulcer. It refused to go near the ulcer.

Experiment 2.—Placed a specimen of the same insect over an infected ulcer. The insect went to the ulcer at once and fed from its secretions, but did not deposit larvæ.

On the following day the feces of this insect were examined and found to contain an average of 7 or 8 acid-fast bacilli per oil-immersion field. No further examination was made until the third day (four days from the time of feeding), when the insect was found dead. Its intestinal contents were examined, but no acid-fast bacilli were found.

Experiment 3.—Another fly of this species was chosen. It lit upon the ulcer, fed freely, and deposited three larvæ. These larvæ were labeled "21–A," and will be referred to later.

Second day: The adult insect was given a clean cage and food.

Third day: Its feces were examined and showed an average of 55 acid-fast bacilli to each oil-immersion field.

Fourth day: Examination of the feces of this insect showed 18 acid-fast bacilli per oil-immersion field.

Fifth day: Examination was made of the feces of this insect and showed an average of 12 acid-fast bacilli to each microscopic field.

Sixth day: Feces of this insect were found to contain an average of between 3 and 4 acid-fast bacilli per field.

Seventh day: The search of the feces showed an average of a little more than 1 acid-fast bacillus to each oil-immersion field.

As the acid-fast bacilli had been reduced to such small numbers on this day, no further examinations of the feces were made.

EXPERIMENTS WITH HOUSE FLIES (*Musca domestica*), THAT WERE FED WITH GRASS BACILLI.

Experiment 1.—First day: Placed 50 house flies (*Musca domestica*) in a large glass jar, covered the latter with cheese cloth, and fed the insects agar-culture of grass bacilli, suspended in bouillon containing sugar.

Second day: Withdrew the bouillon containing the grass bacilli, and fed the insects peptone bouillon containing sugar, but no grass bacilli. (For the sake of brevity we state here that this sterile food was renewed daily throughout the life of the insects, no more acid-fast bacilli being given them).

Third day: Several flies found dead in cage; examined them and found that their intestinal contents showed from 35 to 50 acid-fast bacilli per oil-immersion field. Thirteen specks were examined, these being deposited from the time of the withdrawal of the food to the twenty-fourth hour thereafter—i. e., forty-eight hours from the earliest possible time that the insect could have fed on the acid-fast organisms and twenty-four hours from the last opportunity the insect had of imbibing the bacilli. Four of these were negative as to acid-fast organisms, the balance showing from 2 to 4 acid-fast bacilli per oil-immersion field. Later on the same day eight specks were taken from the jar and examined. Two of these specks showed no acid-fast bacilli, six showed from 25 to 40 acid-fast bacilli per oil-immersion field. Still later, on the evening of the same day, six more specks were examined. Four of these showed no acid-fast bacilli and two contained on an average 45 bacilli per microscopic field.

Fourth day: Thirty-eight specks were examined, and for the sake of convenience from these thirty-eight specks 19 specimens were made—i. e., each specimen stained for examination was composed of two flyspecks. Each one of these nineteen specks contained acid-fast bacilli, the number varying from 1 acid-fast bacillus to 23 acid-fast bacilli per field, the average being 6 acid-fast bacilli per field.

Fifth day: Eighteen flyspecks were chosen and examined. All showed acid-fast bacilli, from 1 to 8 per microscopic field.

Sixth day: Ten specimens of feces examined showed 3 acid-fast bacilli to the field, 14 specimens of feces showed no acid-fast bacilli, 10 specimens examined showed an average of 1 acid-fast bacillus to the field.

Seventh day: Nearly all insects dead. Four of the living ones were killed and their intestinal contents examined. No acid-fast bacilli were found; experiment closed.

Experiment 2.—Placed 49 flies in large cage and fed them with agar-culture of grass bacilli, suspended in bouillon containing sugar.

Second day: Withdrew the grass bacilli; from now on only feeding sterile sugar broth. Divided the insects into six groups and placed each group in a separate cage.

Third day: Killed first group composed of 5 flies (3 having died), smeared their intestinal contents on glass slides, and stained former. First fly showed an average of 1 acid-fast bacillus to two microscopical fields; second insect showed very few acid-fast bacilli, 1 to many fields; third insect showed an average of 80 acid-fast bacilli per microscopical field; fourth insect showed on an average 10 acid-fast bacilli to each field; fifth insect showed an average of 15 acid-fast bacilli per field.

Fourth day: Killed the second group, composed of 7 flies (1 having died). Three of these insects showed no acid-fast bacilli, 1 showed an average of 4 bacilli to each field, 1 showed an average of 10 bacilli to each field, 1 showed an average of 1 bacillus to four fields, and 1 showed 2 bacilli to each field.

Fifth day: Third group killed, composed of 8 flies. Five flies showed no acid-fast bacilli in their intestinal contents, 2 showed an average of 1 acid-fast bacillus to seven fields, and 1 showed an average of 12 bacilli to each field.

Sixth day: Fourth group killed, composed of 6 flies (2 having died). None of these insects showed any acid-fast bacilli.

The insects of the last two groups were destroyed, owing to the failure to discover any acid-fast bacilli in the fourth group.

The preliminary experiments with the grass bacillus show:

First. That certain "blowflies," when given an opportunity, will light upon an ulcer and imbibe of its contents; that by doing this it takes in the bacilli that are present in the secretions of the ulcer, and later frees itself of them by means of its intestinal discharges. While it is not possible with the few experiments we have performed to speak positively on the point, the impressions we gained in this preliminary work were that the bacilli probably did not increase nor were they destroyed in its intestinal tract, but that the number discharged approximated the number imbibed.

Second. That flies of certain species deposit larvæ in ulcers; in fact in some cases appear more anxious to do this than to feed themselves.

Third. That in the "house fly" (*Musca domestica*) we have an insect that will harbor these bacilli in its intestinal tract for several days after imbibing them.

House flies are very numerous in most localities, and while it is our intention to determine how long the bacilli remain in the intestinal tract, we feel that this is not the most important part of the subject, but rather how many bacilli they imbibe and discharge; for, given a constant source of supply, the number of times these insects will fill themselves with such organisms and later discharge them is probably limited only by the length of the insect's life.

Furthermore, its propensity for lighting upon ulcers exposed to its attacks is too well known to require laboratory data on the subject; it is also well known that the same insects light upon the skin and exposed mucus membrane of man, upon his food supply, and in addition to this deposit their feces in all conceivable places, and that such feces afterwards become dry and are blown about as "dust."

In regard to the larvæ above referred to, and designated as "2–A," "13–A," "17–A," "4–A," "12–A," "21–A," for the sake of brevity we shall not describe each of these experiments separately, as they all gave practically parallel results, and show that the larvæ of these insects, which had fed upon these acid-fast bacilli, contain immense numbers of the said bacilli immediately after such feeding. These bacilli, however, progressively decrease in numbers from the time of feeding up to the time the larvæ enters its pupa stage. At the time of this latter event there are nearly always a few acid-fast bacilli still present, but only a very small percentage of the number present on the day of the feeding.

These acid-fast bacilli, and in our experience other micro-organisms, completely disappear before the insect emerges as a fly. The larvæ can, therefore, not be considered as being of importance in the transmission of a bacillary disease; the rare accident of a larvæ as such reaching another human being is so small that we need not consider it.

In the case of larvæ designated as "4–B" (vide supra), we supplied them throughout their larvæ existence with muscle that had been infected with grass bacilli. They were, as would be expected, heavily infected with grass bacilli up to the time they entered the pupa stage, but emerged in the insect stage free from acid-fast bacilli.

That flies of this type do light upon the ulcers of lepers, and do deposit larvæ upon them, and that these larvæ remain for a number of days (and possibly reach the pupa stage) in such ulcers, are matters of clinical observation. Patients have been received at Kalihi whose leprous ulcers were infested with such larvæ. Even with this fact known to us, the manner in which bacteria disappear during the pupa stage so convinced us of the nonimportance of this phase of the subject that we made no attempts to duplicate the larvæ experiments in the work with the bacillus of leprosy, but confined our attention to the adult insect.

PART II. THE BACILLUS OF LEPROSY.

EXAMINATION OF THE ULCERS OF LEPERS FOR LEPRA BACILLI.

We chose 20 ulcers on the bodies of 8 patients under our care; from each of these ulcers we made a smear and stained it in the usual manner. Fourteen of these ulcers contained lepra bacilli in abun-

dance, 3 of them contained a moderate number of lepra bacilli, and 3 contained no lepra bacilli.

It is hardly necessary to state that the ulcers we chose were those formed by the breaking down of leprous nodules, and not the neurotrophic ulcer.

Flies and Human Lepra Bacilli.

Experiment 1.—Twenty house flies (*Musca domestica*) were placed in a cheese-cloth covered jar; the insects were fed a broth suspension containing lepra bacilli, which were obtained from leprous ulcers; this suspension was not rich in bacilli, however, but it was the best we could obtain at the time.

In this cage, containing the 20 flies, we placed a number of glass slides in such a manner as to offer attractive roosting places for the insects, and so arranged them that the flies had access to only one side of each slide. The following day the flies were all found dead (cause of death unknown). We had intended withdrawing the leprous material at this time, but their premature death prevented us from carrying out this part of the experiment. They had, however, deposited 4 specks upon the slides before death. These were stained and examined, 2 of these showing a moderate number of lepra bacilli and the other 2 specks showing no lepra bacilli. Eight of these dead flies were chosen at random and their intestinal contents smeared and examined. Four of these showed no acid-fast bacilli of any kind, 4 showed a large number of acid-fast bacilli identical in appearance with the lepra bacilli, and often present in large clumps.

Experiment 2.—Placed 25 flies in a jar and fed them the scrapings from a leprous ulcer, diluted with normal saline solution and the yolk of an egg, this dilution being necessary on account of the small amount of the scrapings. The resulting suspension was far from being rich in lepra bacilli.

At the end of twenty-four hours many of the insects were dead. They were given fresh slides at that time, and the leprous material was removed from the cage, broth being given them instead.

Twenty-four hours after the withdrawal of this leprous material 16 flyspecks had been deposited on the glass slides. They were stained and examined, with the following results:

16 specks were examined.
4 specks showed no lepra bacilli.
11 specks showed lepra bacilli, usually in considerable numbers, two or three large clumps often being observed in a microscopical field.

Twenty-four hours later all these flies had succumbed but 5. These 5, together with 5 dead ones, were chosen and their intestinal contents examined, and showed that 4 of these insects contained lepra bacilli; 6 insects showed no lepra bacilli in their intestinal tracts.

Experiment 3.—Placed 100 flies in a large jar, gave them undiluted scrapings of leprous ulcers, these scrapings being very rich in lepra bacilli. Hung 14 glass slides in the jar for the insects to roost upon.

Twenty-four hours later about one-half of the insects were dead, the slides were removed, and the numerous specks contained on them were examined in the usual manner. The results of the examination of these specks were as follows:

147 specks were examined.
106 specks showed no lepra bacilli.
41 specks showed lepra bacilli.

In these latter the number of bacilli was simply enormous, the lowest number being present in any one of the 41 specks was 6,300, the largest number 28,000, the average being 17,500 per speck.

Of the 147 specks thus deposited on the slides in the first twenty-four hours, 41 contained a total of 717,500 lepra bacilli. The specks deposited on these slides were but a small percentage of the total number deposited on the other parts of the cage.

The food containing lepra bacilli was now removed and bouillon substituted.

After twenty-four hours more—i. e., forty-eight hours from the time the leprous material was given the flies, and twenty-four hours from the time that it was taken out of the cage, nearly all the insects had died. Four or five remained alive, but rested upon the bottom of the cage and were entirely inactive.

The slides which had hung in the cage from the time the leprous material was removed up to the time the flies had died were withdrawn, but showed only 16 well-defined specks. These were stained in the usual manner and examined:

16 specks were examined.
13 specks showed lepra bacilli.
3 specks showed no lepra bacilli.
Of the 13 specks, the smallest number of lepra bacilli in 1 speck was 250; the largest number, 36,000; and the average number, 12,950.

The experiment was closed here on account of the death of the insects.

Experiment 4.—Placed 100 flies in four cages—i. e., 25 insects to each cage, and fed them fresh, undiluted scrapings from leprous ulcers.

On the following day, of the 100 flies only 12 remained alive (the cause of death unknown). Removed the slides and examined them with the following results:

47 well-defined specks were deposited on the slides during the first twenty-four hours of the experiment.
27 specks showed no lepra bacilli.
20 specks showed lepra bacilli (the average number of bacilli of the 20 specks was approximately 2,200 bacilli per speck).

The leprous material was now removed and the 12 surviving flies placed in one cage and given sterile bouillon as food.

Twenty-four hours later only 6 of these insects were alive. By chance no feces had been deposited on the glass slides, so no examination of the feces was made. The 6 dead flies were smeared and examined. The intestinal contents in each fly showed a moderate number of lepra bacilli; no count of the number of bacilli per smear was made.

Twenty-four hours later—i. e., three days from the beginning of the experiment, only 2 flies remained alive, no specks being deposited upon the glass slides. These two insects were killed with chloroform and examined, but no lepra bacilli were found in their intestinal contents.

Experiment 5.—Placed 100 flies in a cage; gave them undiluted scrapings of leprous ulcers; also gave them broth containing sugar, in hopes of prolonging their lives, in order to ascertain more definitely than the previous experiments have shown how long the lepra bacilli are present in the intestines.

Twenty-four hours later found most of the insects alive. These were removed to another cage; the specks on the slides, which latter had been hanging in the former cage for the first twenty-four hours of the experiment, were stained in the usual manner. They showed 34 well-marked specks.

34 specks were examined.
11 specks showed no lepra bacilli.
23 specks showed lepra bacilli (the number varying from 20 bacilli to 30,000 bacilli per speck, the average number per speck being 2,160 bacilli).

Inasmuch as it was believed that the manipulation necessary for the transfer of these insects might be responsible for the mortality they were allowed to remain three days after the above transfer without being disturbed—i. e., four days from the beginning of the experiment. They were given fresh food and slides.

Twenty-four hours after these latter slides were placed in the cage—i. e., five days from the beginning of the experiment, and four days from the removal of the leprous material, 17 flies still survived, and 21 specks had been deposited upon the slides hung in the jar.

21 specks were examined.
19 specks showed no lepra bacilli.
2 specks showed lepra bacilli (1 of these specks contained 14 lepra bacilli, and the other speck contained 175 lepra bacilli).

On the afternoon of the same day the 17 flies remaining alive were killed with chloroform and examined. Their intestinal contents showed no lepra bacilli, they having freed themselves of them in four days and a few hours from the time they had had the last opportunity to feed upon the bacilli, and five days and a few hours from the time they had the first opportunity to feed on the bacilli.

Experiment 6.—Placed 100 house flies (*Musca domestica*) in a jar, gave them the undiluted scrapings of a leprous ulcer, also (separately) sugar broth. The flies at first lit upon the leprous scrapings and fed from them freely, for the time being neglecting the broth. They were furnished clean slides, as in the previous experiments.

Twenty-four hours later the leprous material was taken away from them and more sterile sugar broth placed in a clean cage, into which the insects were transferred. The slides, that had hung in the cage from the time that the leprous material was fed up to the time the same material was withdrawn—i. e., twenty-four hours during the first experiment, were removed; the specks contained on these slides were stained and examined with the following results:

61 specks were examined.
49 specks showed no lepra bacilli.
12 specks contained lepra bacilli.

These 12 specks containing lepra bacilli showed these organisms to be present in immense numbers, the average number of lepra bacilli per speck being 18,000; 1 speck showed over 32,000 lepra bacilli.

At the end of another twenty-four hours the insects were again removed to a clean cage, given fresh, sterile food and clean slides. The old slides, which had hung in the jar from the time that the leprous material was taken from the flies up to twenty-four hours following this event, were removed, and the specks stained and examined with the following results:

78 specks were examined.
75 specks showed the presence of lepra bacilli.
3 specks showed no lepra bacilli.
The average number of lepra bacilli per speck was 13,500.

Twenty-four hours later the flies were again transferred to a cage, supplied with fresh, sterile food and clean slides. The old slides were removed and their specks stained and examined with the following results:

86 specks were examined.
54 specks showed lepra bacilli.
32 specks showed no lepra bacilli.
The average number of lepra bacilli per speck was 887.

Twenty-four hours later the insects were again transferred to a clean cage and furnished with sterile sugar broth and fresh slides. The old slides were removed and the specks contained on same stained and examined with the following results:

47 specks were examined.
17 specks showed lepra bacilli.
30 specks showed no lepra bacilli.
The average number of lepra bacilli per speck (considering the 17 specks only) was 32.

Twenty-four hours later the insects were transferred to another cage and furnished with sterile food and clean slides. The old slides

were removed and the specks contained on same stained and examined with the following results:

34 specks were examined.
No lepra bacilli were found in any of the specks.

Twenty-four hours later about 50 of the original 100 flies were still alive. These were killed with chloroform; 39 of these insects were chosen, their intestinal contents smeared on glass slides, stained, and examined; no acid-fast bacilli were found in their intestinal tracts.

Experiment 7.—Placed 100 house flies (*Musca domestica*) in a clean jar and gave them glass slides to roost upon and sterile sugar bouillion for food. Into this jar we introduced scrapings of a leprous ulcer, rich in lepra bacilli.

On the following day, i. e., twenty-four hours from the beginning of the experiment, the leprous material was withdrawn, the insects were transferred to a clean cage, and furnished sterile sugar broth for food. The old slides were taken out of the former cage and clean slides hung in the new cage. The specks contained on the old slides were stained and examined with the following results:

185 specks were examined.
116 specks showed lepra bacilli.
69 specks showed no lepra bacilli.

No count of the number of lepra bacilli per speck was made, but the average ran high into the thousands.

Twenty-four hours later, i. e., twenty-four hours from the time the leprous material had been withdrawn from the flies, the insects were changed to another clean cage and given fresh food and clean slides. The old slides were removed, but not examined on account of the pressure of other work.

Twenty-four hours later the insects were changed to another clean cage and given clean slides and fresh sterile food. The old slides that had hung in the old cage for the last twenty-four hours were removed, but for the reason stated in the last experiment were not examined.

Twenty-four hours later, i. e., seventy-two hours from the time the leprous material was taken away from the flies, the insects were changed to another clean cage, and the slides that had hung in the cage from the forty-eighth to the seventy-second hour from the time that the leprous material had been withdrawn were stained and examined with the following results:

81 specks were examined.
34 specks showed lepra bacilli.
47 specks showed no lepra bacilli.
Considering the 34 specks alone, the average number of lepra bacilli per speck was 673.

Twenty-four hours later, i. e., ninety-six hours from the time that the leprous material was taken away from the flies, the insects were transferred to another clean cage and furnished with fresh slides and

sterile food. The old slides which had hung in the cage from the seventy-second hour to the ninety-sixth hour from the last opportunity the insects had had for feeding on the leprous material were removed, stained, and examined, with the following results:

32 specks were examined.
11 specks showed lepra bacilli.
21 specks showed no lepra bacilli.
The average number of lepra bacilli per speck (the 11 specks alone considered) was 93.

Twenty-four hours later we removed the flies to another clean cage, supplied them with fresh food and slides, removed the slides which had hung in the cage for the previous twenty-four hours, and stained and examined them with the following results:

27 specks were examined.
No lepra bacilli were found in any of the specks.

The insects were on the same day killed by means of chloroform, 39 having still survived out of the original 100. The bodies of 17 of these were chosen, their intestinal contents smeared on glass slides, stained, and examined. No lepra bacilli were found in any of the insects.

Lepra Bacilli and *Sarcophaga barbata*.

Captured 5 specimens of *Sarcophaga barbata* and placed each in a bottle; made an artificial ulcer, in a manner previously described, on the abdomen of a rabbit; made a suspension of lepra bacilli (but from the material at hand the suspension was poor in these organisms) and filled the ulcer with it.

Experiment 1.—The first insect was placed over the ulcer. It lit near its border and appeared to feed, but of this we could not be certain, as it was on the ulcer but an instant. Fifteen minutes after it had lit on the ulcer it was killed with chloroform and examined, but no lepra bacilli were found. The insect did not deposit larvæ.

Experiment 2.—Four insects were placed separately, one after the other, over the same ulcer; all lit near the ulcer, but remained there only for an instant. The insects apparently fed a little, but of this we could not be certain.

The hesitation of these insects to partake freely of the food offered to them may have been due to the employment of a hair-removing preparation instead of a razor on the abdomen of the rabbit previous to the experiment. None of these insects deposited any larvæ on the ulcer's edge, but 3 of them deposited larvæ freely after being placed in their cages. Fifteen minutes after these insects had apparently fed they were destroyed with chloroform; 2 of them showed no lepra bacilli in their intestinal contents, while 2 of them showed a large number.

Experiment 3.—Took 1 specimen of *Sarcophaga barbata* and placed it over an artificial ulcer made in the skin of the abdomen of a guinea pig and infected with the scrapings of a human leprous ulcer. The insect fed, but did not deposit larvæ.

The following morning the fly was still alive and was killed with chloroform. A smear of its intestinal contents was made, stained, and examined. It showed a number of lepra bacilli, on an average 30 to an oil-immersion field.

During the night following the feeding this insect had deposited 6 specks. These 6 specks were stained and examined and all found to contain lepra bacilli, the average being something over 1,100 lepra bacilli per speck.

Experiment 4.—Placed 12 specimens of *Sarcophaga barbata* over an artificial ulcer made in the skin covering the abdomen of a guinea pig, and which had been infected with human lepra bacilli obtained from the scrapings of an ulcer of a case of human leprosy. Seven of these insects were seen to light on the ulcer and appeared to feed; 5 of them would not feed. These insects were killed with chloroform at the end of eighteen hours and their intestinal contents smeared; 5 of them showed large numbers of lepra bacilli in their intestinal contents, while the others showed none. During the eighteen hours mentioned, preceding the death of the insects, they had deposited a number of specks. Twelve of these specks were chosen and examined with the following results:

12 specks were examined.
9 specks showed lepra bacilli.
3 specks showed no lepra bacilli.

Experiments with the Undetermined species of *Lucilia*.

Experiment 1.—In the manner previously described we made an artificial ulcer and infected it with the scrapings of a leprous ulcer. Placed 8 specimens of *Lucilia* over this ulcer; 3 apparently fed on this ulcer, and 5 of them did not feed.

On the following day the flies were killed and examined. Only 1 of these insects showed lepra bacilli in its intestinal contents, and this one contained exactly 98 in its whole intestinal tract.

Two specks were taken out of the cage (most of them were inaccessible, having been deposited on the coverings of the cage) and examined with the following results:

2 specks were examined.
1 speck contained 168 lepra bacilli.
1 speck contained over 1,800 lepra bacilli.

Comment: It would appear, from this experiment, that this *Lucilia* frees itself of its intestinal contents more rapidly than do the other insects. This would account for the peculiar result just noted.

Sarcophaga pallinervis.

No experiments were attempted with *Sarcophaga pallinervis* for two reasons—one, that it seemed it would have been an unnecessary repetition of the *Sarcophaga barbata's* experiments, these insects being closely allied; the other reason being that the sanitary regulations of the station have been so strictly enforced that all varieties of flies have become very scarce, and the *Sarcophaga pallinervis* has practically disappeared.

DISCUSSION.

The above data, taken together with the well-known habits of certain of these flies, establishes the truth of the following statements:

First. That given a bacilli-discharging leprous ulcer (and practically all leprous ulcers due to the breaking down of a leprous nodule do discharge bacilli), unprotected from the attacks of flies, these insects will convey the bacillus of leprosy to the skin of near-by healthy persons.

Second. That such insects will contaminate the foodstuffs of such persons with these organisms.

Third. That these insects deposit their feces on every conceivable surface, and such feces, becoming dry and pulverized, will in the form of "dust" convey the bacilli to the nasal mucosa of near-by persons.

That given such an unprotected ulcer, and the usual number of flies, this contamination of the skin and mucosa of near-by healthy persons will not be an unusual event, but will occur again and again, through an indefinite length of time, and the number of bacilli thus conveyed to healthy persons will be enormous.

This much the experiments show; but the below-mentioned points at once present themselves, and can not in our present state of knowledge be answered by laboratory data:

First. Is the skin (or abrasion of it), the mucosa of the digestive tract, or the nasal mucosa capable of being infected with leprosy?

Second. Are the bacilli that have been discharged from an ulcer and afterwards passed through a fly's digestive tract alive and capable of infecting man?

Third. Do epidemiological and clinical observations bear out the supposition that flies help to spread leprosy?

As to the first of these, we know that by some means lepra bacilli *do* gain entrance to the tissues of man. It is difficult to conceive how this is brought about, except that the bacilli penetrates one or more of the surfaces mentioned as being frequently contaminated, directly or indirectly, by the fecal contents of flies.

As to the second, on this we have very little data. In the well-known case of Arning [a] the prisoner Keanu was inoculated with a leprous nodule that had not broken down into an ulcer. The patient developed leprosy, but he had been in contact with lepers in his own family and in prison (Swift). Furthermore, he was a Hawaiian. The mathematical probabilities of one of these people developing leprosy under ordinary conditions is (and was at the time of the experiment) high.

Bearing on this we also have the case of Coffin,[b] in which a prisoner purposely inflicted a wound of the skin and inoculated himself with leprosy, employing in this case as material the pus of a leprous ulcer. The man developed leprosy, but he had, before inoculating himself, been exposed to lepers.

On the other hand, many alleged and authentic cases of human inoculation, performed usually by means of a wound of the skin, have failed to reproduce leprosy. In weighing this evidence, however, it must be remembered that much negative evidence is required to outweigh a very few positive results in a matter of this kind.

Bearing indirectly on this question, we know that in rat leprosy, a disease closely resembling human leprosy, the inoculation of an abrasion of the skin of a healthy rat with the bacilli contained in an ulcer of a leprous rat is usually followed by infection.

As to the third of these questions, Do epidemiological and clinical data bear out the supposition that flies help to spread leprosy? The writer, in connection with another work, has had occasion to review recently a large mass of data and opinions contained in the literature bearing on the general subject of transmission of leprosy. The most striking point that appears in much of the literature is the large number of lepers that give a history of personal contact of the most intimate character with a leper (for a long or short period) previous to developing the first symptoms of the disease. Examples could be cited that would seem to show that those persons who reside in the same house with a leper, but do not eat or sleep with or administer to the patient, are much less often attacked than persons who, in addition to residing with the afflicted person, also come in very intimate contact with him.

---

[a] For details of this case, see The case of inoculated leprosy, by Arning, Dermat. Congress, Prague, Wiener med. Wochenschrift, No. 29; Editorial Lancet, July 27, 1889, p. 184; Scheube: Diseases of Warm Countries; also report of Emerson and Kimball in Tebb's The Recrudescence of Leprosy, p. 123; also Swift and another article by Swift and Montgomery, both reviewed by Baumgarten, vol. 6.

[b] Etude de la lèpre aux îles de Maurice et de la Réunion. Scheube: Diseases of Warm Countries.

This, of course, is against transmission by an insect having a considerable excursion of flight, like the fly. This observation is not strongly against household infection, for the probabilities of the fly carrying the infection to some one within the household are far greater than those of the insect carrying the infection to a near-by dwelling; but it is distinctly against fly-borne infection that a person sleeping in the next room should be quite exempt, while the person sleeping in the same room is apt to be infected. We do not admit such is the case, however, although the experience of many would appear to indicate this.

In considering the matter, it should be remembered that it is improbable than any *one* insect, mechanism, or means is alone responsible for the spread of leprosy; furthermore, that while observations of a clinical and epidemiological character should be given due weight, it must be remembered that data of that kind has often led to errors.

CONCLUSIONS.

First. That the above-named flies, when given an opportunity to feed upon leprous fluids, will contain the bacilli in their intestinal tracts and feces for several days after such feeding.

Second. That the above fact, together with the well-known habits of these flies, make it certain that, given an exposed leprous ulcer, these insects will frequently convey immense numbers of lepra bacilli, directly or indirectly, to the skins, nasal mucosa, and digestive tracts of healthy persons.

Third. That our present state of knowledge does not permit us to determine whether such insect-borne bacilli are or are not capable of infecting persons whose skin and mucosa are thus contaminated; but until we have more accurate knowledge on this point we are justified in regarding these insects with grave suspicion as being one of the means of disseminating leprous infection.

HEREDITY VERSUS ENVIRONMENT IN LEPROSY.

By Harry T. Hollman,
Acting Assistant Surgeon, Leprosy Investigation Station, Public Health and Marine-Hospital Service.

In 1885 the Board of Health of Hawaii established a home in Honolulu for nonleprous girls born of leprous parents. It was not until 1908, under Dr. L. E. Cofer, U. S. Public Health and Marine-Hospital Service, then acting president of the Board of Health of Hawaii, that a home for nonleprous boys born of leprous parents was established.

Therefore, prior to 1908 the boys born at the Leper Settlement on Molokai were exposed to the disease for a longer average period than were the girls born there.

Having a knowledge of these facts, studies were undertaken by me in order to ascertain the influence of heredity and environment in the causation of leprosy.

For a number of years it has been said that "a very small percentage of the number of children born of leprous parents in Hawaii developed leprosy if removed early from exposure."

Fitch (1) said: "In eighteen years only twenty-six children were born, and only two of these became lepers in Molokai."

James C. White (2) has also said: "But two children born in Molokai have contracted the disease."

These and many other assertions regarding the birth rate of children born of leprous parents and the subsequent development of leprosy among them are incorrect, as accurate data could not have been obtained. For example, in the biennial report of the president of the Board of Health of Hawaii, ending 1892, the superintendent of the Leper Settlement, in giving the number of children born to lepers, writes: "There is probably an error in this figure."

In studying the data collected the following points have been considered: First, whether or not the length of exposure was in direct ratio to the number of cases of leprosy developing in children born of leprous parents; second, if heredity per se is a factor in the causation of leprosy.

In order to obtain material for this study the following records were carefully searched: Records of births and deaths in the Leper Settlement since 1896, when registration was made compulsory; records of declared lepers; records of nonleprous boys' home; records

of nonleprous girls' home; records of the superintendent's office at the Leper Settlement since 1880.

The results are tabluated in three groups: Group I, male and female children born of leprous parents, whose life histories to July 31, 1909, are known, and who were under observation ten or more years; Group II, male children born of leprous parents, whose periods of exposure are known, and who were under observation at least seven years; Group III, female children born of leprous parents, whose periods of exposure are known, and who have been under observation at least seven years.

GROUP I.

From January 1, 1896, to July 31, 1909, 262 children were born of leprous parents, 128 being males and 134 females. Of the males, 51 died (nearly 40 per cent) under one year; of the females, 56 died under one year (41 per cent).

There were under observation for ten or more years, 22 males and 24 females. The length of exposure of the 22 males was ten or more years; the lengths of exposures of the 24 females were as follows:

One year or less	4	Six years	2
Two years	5	Seven years	4
Three years	2	Eight years	1
Four years	4	Twelve years	1
Five years	1		

The minimum time of exposure was eighteen days, the maximum time of exposure was twelve years, and the average time of exposure was four and seventeen twenty-fourths years.

Of the 22 males continuously exposed ten or more years, 7 contracted leprosy at the following ages: One at 4 years (father and mother advanced tubercular lepers), 3 at 6 years, 2 at 7 years, and 1 at 9 years. The average age at which leprosy was contracted was 6¼ years.

Of the 24 females whose average exposure was four and seventeen twenty-fourths years, 1 contracted leprosy. This case was removed from exposure at the age of 3 years.

GROUP II.

Thirty male children born of leprous parents, whose periods of exposure are known, and who were under observation ten or more years. They were exposed as follows:

Twenty-three years	1	Fourteen years	6
Twenty years	1	Eleven years	3
Nineteen years	2	Ten years	1
Eighteen years	1	Eight years	5
Seventeen years	3	Seven years	3
Fifteen years	4		

The maximum time of exposure was twenty-three years, the minimum time of exposure was seven years, and the average time of exposure was thirteen and twenty-six one-hundredths years. Of these 30 cases 3 (or 10 per cent) contracted leprosy, 2 at the age of 15 years and 1 at the age of 7 years.

GROUP III.

Seventy-eight female children born of leprous parents, whose lengths of exposure were known, and who were under observation seven or more years. Their exposures were as follows:

Seventeen years	1	Eight years	7
Sixteen years	1	Seven years	8
Fifteen years	2	Six years	2
Fourteen years	3	Five years	2
Thirteen years	5	Four years	5
Twelve years	5	Three years	7
Eleven years	2	Two years	7
Ten years	7	One year	8
Nine years	6		

The maximum time of exposure was seventeen years, the minimum time of exposure was one year, and the average time of exposure was seven and one-half years.

Of these 78 cases 12 contracted leprosy, after the following lengths of exposure:

After exposure of—	
Seventeen years	1
Thirteen years (developing disease in her fourteenth year)	1
Thirteen years	1
Twelve years	1
Nine years	1
Eight years	1
Seven years (contracted at 7 years)	3
Four years (developing disease at 11 years)	1
Two years (developing disease in her tenth year)	1
One year (developing disease in her seventh year)	1

The average time of exposure was eight and thirty-six one-hundredths years. In the 4 cases of which we have accurate data, the average time between cessation of exposure and detection as a leper was five and a half years.

SUMMARY.

I. It is shown that 40 per cent of the children born of parents one or both of whom were lepers died under one year.

II. Thirty-two per cent of the males who were exposed ten or more years developed leprosy.

III. Four per cent of the females whose average time of exposure was less than five years developed leprosy.

IV. Ten per cent of the males exposed for more than seven years developed the disease.

V. Thirteen per cent of the females exposed from one to seventeen years and under observation seven or more years became lepers.

VI. The average time of exposure of the cases which developed leprosy was five years.

DISCUSSION.

The high death rate among children born of leprous parents is what one would expect to find. The parents, being the battleground of a conflict between the lepra bacilli and their tissues, would not be expected to produce strong, healthy children.

A comparison of the number of male children who were exposed ten or more years and developed leprosy (32 per cent) with the number of female children who were exposed less than half as long and developed the disease (4 per cent) would tend to show that the danger of contracting leprosy for children born of leprous parents is in direct ratio to the length of the exposure.

The results presented under Group I, although based on a total of only 46 cases who survived the first year and became long exposures (males) and short exposures (females), are convincing, because the data are complete and further substantiated by the summaries presented in Groups II and III.

It is evident from the data contained in Groups II and III that a much higher percentage of the female children who were exposed for a long period of time to the disease became lepers than the males (13 to 10). According to Brinckerhoff (3), the ratio in Hawaii of the sexes of those who are declared lepers is 65½ per cent males to 34½ per cent females.

The difference in our group averages may be due to one or more causes.

The number of females (78) in Group III may be too large to compare accurately with the males (30) in Group II, or, as seems far more plausible, female children, born of leprous parents when under long exposure to the disease, are from their sex more in direct contact with leprous contagion, i. e., household duties, washing clothes, etc., while boys are, as any other boys, more out of the house than in it, hence in much less direct contact with the contagion.

Notwithstanding this difference, Groups II and III give emphasis to the fact that the longer the exposure the more cases of leprosy develop in the children.

In the opinion of many authorities leprosy is contracted by contagion only. Impey (4) says, "Leprosy is spread by contagion only." Thompson (5) has written, "Infection is received by man from his environment rather than from the sick" (house infection). Pernet (6), in speaking of the Purulia conference, says, "The delegates arrived at an unanimous opinion that leprosy is contagious," while Donovan (8) quotes Kaposi as follows: "Lepra has never been

observed in the children of leprous parents immediately or within a short period after birth." Espinet (13) wrote that he had never seen it in children under 3 or 4 years of age. Castor (12), according to Thin, had seen no case of congenital leprosy. Sanddreczki (14), in A Study of Leprosy, said he had never observed it in the newly born. The leprosy commission in India (11), in their report, said, "No case of congenital leprosy was seen." To quote from a lay authority on lepers, Mr. Jackson (15) of the mission to lepers in India and the East, in his book In Leper Land, says: "At one of the Indian missions, previous to the establishment of a home for the children of lepers, the former lived with their parents continuously, and as a result most of them developed leprosy; in fact, Reverend Guilford states of these children (before the establishment of a separate home): 'Of all those born there during the last thirty years I know of only two men who have not become confirmed lepers, and even these, when I last saw them, began to show signs of the disease. * * * while during the same period (thirty years) at another asylum near by means of separating the children have existed and among these only 1 case of leprosy has developed.'"

The leprosy commission in India in 1890 found that "of children born of leprous parents only a very small number became leprous; thus 98 leprous couples had 65 children, and of these 3 became lepers. Among 500 children born of two leprous parents, or from one leprous parent and one healthy parent, 21, or 4.2 per cent, became lepers." They also state, "Leprosy is certainly not found as often as in 1 out of 100,000 cases at an infantile period."

This is, in fact, an argument for the transmission of the disease by contact, as our observations and those of others show that a certain percentage of children, when exposed, will contract the disease.

Therefore the sooner after birth a child born of leprous parents is removed from exposure the less likelihood is there of its subsequently developing leprosy.

From a study of those of our cases who developed leprosy, and of whom there are accurate data, it is learned that the average time of exposure was five years, and that the disease was on an average five years in developing after the cessation of exposure.[a]

Although many authorities cite cases born with recognizable lesions, in our studies of the 262 cases we found no record of any child having recognizable lesions of leprosy at birth. Reschetillo (7) mentions 28 children born of leprous parents, 3 of whom had recognizable lesions. Navarro (16) has reported 2 cases of congenital leprosy. Hansen (9) has reported having found lepra bacilli in the

[a] There may be an unavoidable error here, as the disease may have been far advanced when they were pronounced lepers. Our data simply state the time they were officially declared lepers.

seminiferous tubules of the testicle, and Babes, according to Babs (10), has found lepra bacilli in semen with living spermatozoa, and in the egg sack of the ovary, while Arning (10), according to the same author, has found lepra bacilla in the ovary. Nevertheless, heredity apparently played no part in the causation of our cases.

CONCLUSIONS.

First. The danger of contracting leprosy for children born of leprous parents increases with the length of exposure.

Second. Heredity is not an important factor in the causation of leprosy.

BIBLIOGRAPHY.

1. Fitch, 1894. Baumgarten's Jahresbericht, vol. 7, p. 277.
2. White, J. C., 1894. The contagiousness and control of leprosy. Boston Medical and Surg. Journal, October 25, 1894. (Reprint.)
3. Brinckerhoff, W. R., 1908. The present status of the leprosy problem in Hawaii. Publication of the U. S. P. H. and M. H. Service.
4. Impey, S. P., 1897. Proceedings, Lepra Conference, 1897, p. 94.
5. Thompson, J. A., 1908. Notes on leprosy. Lepra, vol. 7, p. 211.
6. Pernet, George, 1909. The Purulia conference. Lepra, vol. 8, p. 202.
7. Reschetillo, 1903. Ueber die Rolle der Heredität bei der Lepra auf Grund einiger Beobachtungen an Neugeborenen. Baumgarten's Jahresbericht, vol. 19, p. 341.
8. Donovan, Justin Foley, 1897. Proceedings, Lepra Conference, 1897, pt. 4, p. 57.
9. Hansen, A., 1903. Lepra, vol. 3, p. 260; also Baumgarten's Jahresbericht, vol. 7, p. 277.
10. Babs, Hans, 1909. Berlin. Die luetische Infektion in der Schwangerschaft und ihre Bedeutung für das Vererbungsproblem der Syphilis. Centralbl. f. Bakt., Originale, Bd 51, Heft 3.
11. The Leprosy Commission in India, 1890, Report. Lancet, 1893, April 23, p. 1024.
12. Castor, Doctor. Surgeon-general's report, Guiana, 1887. Thin's Text-book on Leprosy, 1891, p. 132.
13. Espinet, Doctor. Leprosy in Trinidad. Lancet, July 8, 1876, p. 74.
14. Sanddreczki, Max. A study of leprosy. Étude sur la lèpre. Résumé des observations faites pendant un séjour en Palestine. Revue Médico-Pharmaceutique. Constantinople, April 30, 1889. Lancet, August 31, 1889, p. 423.
15. Jackson, J. In Leper Land. London, 1901. Reviewed by Lepra, vol. 3, p. 186.
16. Navarro, Doctor. In Revista Médica de Bogotà, November 1, 1890; quoted by Thin in Text-book on Leprosy, 1893, p. 132.

OTHER LITERATURE REVIEWED.

Neumann, Doctor, 1876. Norwegian lepra. Allg. Wien. med. Zeitsch., March, 1876; also Lancet, May 6, 1876, p. 693.

Neisser, 1881. Weitere Beitrage zur Aetiologie der Lepra. Virchow's Archiv, Bd 84, p. 514.

Hillis, John D., 1883. Text-book. Leprosy in British Guiana; an account of West Indian leprosy. Editorial. Lancet, March 31, 1883, p. 556.

Munro, W., 1883. Leprosy. Lancet, May 5, 1883, p. 807.

Cullen, P., 1884. Data on previous histories of 38 lepers. No title. Indian Medical Journal, July, 1884; Lancet, August 30, 1884, p. 380.

Bidenkap, J. L., 1886. An abstract of lectures on lepra. Referat. Fortschr. d. Med., 1886, p. 806; also Baumgarten Jahresbericht, vol. 2, p. 255.

de Aravedo Lima and Guedes de Mello, 1887. Ueber das Vorkommen der einzelnen Lepraformen, sowie der Erscheinungen an Auge, Nase und Ohren. Baumgarten's Jahresbericht, vol. 3, p. 231.

Hansen, A., 1888. Die Erblichkeit der Lepra. Baumgarten's Jahresbericht, vol. 4, p. 221.

Hansen, A., 1890. Ist die Lepra eine im Aussterben begriffene Infektionskrankheit und ist sie erblich? Virchow's Archiv, Bd. 120, p. 476.

Heidenstam, Doctor, 1890. Leprosy in Cyprus. Lancet, April 19, 1890, p. 871.

Thin, George, 1891. Text-book on leprosy, 1891, pp. 136–172.

Arning, E., 1891. Lepra mit besonderer Berücksichtigung der Uebertragung durch Heredität oder Contagion. Arch. f. Dermatol. u. Syphil., Bd. 23, p. 5, 9.

Bergmann, A., 1891. Zur Contagiosität der Lepra. Baumgarten's Jahresbericht, vol. 8, p. 270.

Zambaco Pascha, 1892. Die Lepra ist eine erbliche Krankheit. Baumgarten's Jahresbericht, vol. 10, p. 315.

Buret, F., 1892. Zwei Worte über Lepra. Ist sie erblich oder ist sie ansteckend? Baumgarten's Jahresbericht, vol. 8, p. 270.

Engel, F., 1893. Bericht über eine Lepra-Enquête in Aegypten. Lepra, vol. 3, p. 224.

Babes, V., and Kalindero, 1895. Note sur la distribution du bacillus de lèpre dans l'organisme. Compt. rend. de la Soc. de Biol., p. 629; also Baumgarten's Jahresbericht, vol. 11, p. 323.

Koebner, Doctor, 1896. Discussion of Berlin Med. Soc., 1896. Lancet, December 5, 1896, p. 1637.

Grossmann, Karl, 1896. On the causation of leprosy and the extent of its occurrence in Europe. Lancet, October 17, 1886, p. 1083.

Editor, 1896, Lancet. (Berlin correspondent.) Lancet, May 23, 1896, p. 1460.

During, E. von, 1897. Die Rolle der Erblichkeit. Lepra-Konferenz zu Berlin, 1897, Bd. 2, p. 107.

Hellat, P., 1897. Bemerkungen zur Frage der Heredität. Berlin Konf., 1897; also Baumgarten's Jahresbericht, vol. 13, p. 492.

During, E. von, 1897. Lepra in der Türkei. Lepra-Konf., 1897; also Baumgarten's Jahresbericht, vol. 13, p. 498.

Dohi, K., 1897. Ueber die Lepra in Japan. Lepra-Konf. zu Berlin, 1897; also Baumgarten's Jahresbericht, vol. 13, p. 507.

Besnier, E., 1897. Rôle étiologique: 1. de l'hérédité; 2. de la transmissibilité. Lepra-Konf. zu Berlin, Bd. 1, Abt. 1, p. 127.

Baelz, E., 1897. Zur Lehre von der Lepra und ihrer Behandlung. Berl. klin. Wochenschrift, 1897, pp. 997, 1331.

Alvarez, L. J., 1897. Proceedings of Lepra Conference, 1897.

During, E. von, 1898. Zur Lehre von der Lepra: Contagion und Heredität. Deutsch. med. Wochenschrift, vol. 24, pp. 316, 433.

Zambaco Pacha, 1898. Communication sur la contagiosité de la lèpre. Baumgarten's Jahresbericht, vol. 14, p. 426.

Zambaco Pacha, 1898. Progéniture des lépreux. Lepra-Konferenz zu Berlin, Bd. 3, Abteil. 2, p. 591.

Goldschmidt, Jiles, 1899. La lèpre: Observations et expériences personnelles. Soc. d'Éditions Scientifiques, Paris, 1894; also Lancet, Sept. 30, 1899, p. 885.

Woit, O. R., 1899. Untersuchungen über Lepra-Bacillen in den Organen eines 5 Wochen alten Kindes lepröser Eltern. Baumgarten's Jahresbericht, vol. 15, p. 374.

Glueck, Leopold. 1900. Die Lepra in knapper Darstellung. Abstract in Lepra, vol. 1, p. 217.

Editor, 1901, Lancet. (India correspondent.) Lancet, Oct. 19, 1901, p. 1080.

Kynsey, Sir W., 1902. Proceedings, Royal Medical and Chirurgical Society, London, June, 1902. Lancet, June 14, 1902, p. 1691.

Hansen, G. A., 1902. Proceedings, Royal Medical and Chirurgical Society, London, June, 1902. Lancet, June 14, 1902, p. 1691.

Tonkin, T. J., 1902. On some general and etiological details concerning leprosy in the Soudan. Lancet, May 31, 1902, p. 1535.

Hansen, Armauer, 1902. At a meeting of the leprosy committee in London, 1902. Extract in Lepra, vol. 3, p. 261.

Ashmead, A. S., 1902. Is contagion transmitted by the milk of mothers? St. Louis Medical and Surgical Journal, July, 1902; also Baumgarten's Jahresbericht, vol. 18, p. 350.

Paraskevas, 1903. Contribution à l'étude de la lèpre (étiologie de la lèpre). Thèse d'Athènes, 1902. Lepra, vol. 3, p. 181.

Editor, 1903, Lancet. Editorial, Lancet, June 6, 1903, p. 1603.

Sandwith, F. M., 1903. Meeting British Medical Assn., 1903. Lancet, August 22, 1903, p. 544.

Tonkin, T. J., 1903. An analysis of 220 cases of Sudanese leprosy. Lancet, April 18, 1903, p. 1077.

Dyer, I., 1904. Leprosy from a sanitary standpoint. Lepra, vol. 4, p. 281.

Turner, George. 1904. The ætiology of leprosy. Lepra, vol. 4, p. 126.

Noël, L. A., 1904. La lèpre; douze années de pratique à l'hospice des lépreux de la Désirade (Guadeloupe). Lepra, vol. 4, p. 217.

Black, R. S., 1906. Remarks on leprosy in Cape Colony. Centralbl. f. Path. u. path. Anat., Bd 17, p. 476.

Balvey, Bas., 1907. Sur l'hérédité de la lèpre. Lepra, vol. 7, p. 131.

Zambaco Pacha, 1909. L'hérédité de la lèpre. Paris, Masson, 1908, M. 1–80. Lepra, vol. 8, p. 187.

Blaschko, A., 1909. Das Wiederaufleben der Lepra in Mitteleuropa und seine Ursachen. (Ref. Münch. med. Woch., vol. 57, p. 4, January 10, 1910, No. 1, p. 39.)

O

RA
421
P78
no.38-
39
cop. 2

Public health bulletin

Biological
& Medical
Serials

PLEASE DO NOT REMOVE
CARDS OR SLIPS FROM THIS POCKET

UNIVERSITY OF TORONTO LIBRARY